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THREE LECTURES

ON

EXPERIMENTAL EMBRYOLOGY

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THREE LECTURES
ON
EXPERIMENTAL EMBRYOLOGY

BY

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WITH A BIOGRAPHICAL NOTE

BY

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TO P. FROM Φ. T.

IN MEMORY

PREFACE

THE manuscript of these lectures was found amongst my husband's papers. He had, I know, intended to publish them, but had laid them aside to await final revision before going to press. In these circumstances the best course seemed to be to publish the text exactly as he had left it, while adding a few illustrations to take the place of the lantern slides used at the lectures when these were delivered at University College, London.

I am glad to have the opportunity of acknowledging here my deep indebtedness to all who have so kindly helped me in seeing this book through the Press. My thanks are especially due to Miss Kirkaldy, to Mrs. Cuthbert Baines, to Dr. Marett, to Professor Ramsay Wright, and to the Delegates of the University Press, who one and all by their valuable help and kindly sympathy have made it possible for me to carry out one of my husband's last wishes.

CONSTANCE JENKINSON.

OXFORD.

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JOHN WILFRED JENKINSON

To remember is, during this devastating war-time, almost the sole part left to Oxford. 'How doth the city sit solitary that was full of people! how is she become as a widow!' Of the fallen, most will be remembered as gallant youths who laid down life while it was yet all promise, a garden of blossoming hopes. But some were senior men, with their faculties already mature, and their life-work in active process of achievement. Among such elder sons of the University who fought and are gone was John Wilfred Jenkinson. His name will live on in his written works; for at least something of the rich fruitage of his mind has been harvested, though how much more has been lost can never be known. Yet a man is more than his books. So let these few lines be dedicated to the memory of the man himself—by one who, as his tutor first and his colleague afterwards, learnt to know him for the noble spirit that he was, and to admire and esteem him accordingly.

A contrast is sometimes drawn between the man of thought and the man of action; but it is possible for these diverse characters to unite harmoniously in the same individual. Jenkinson's outstanding quality was strength of purpose. Nothing in the form of work could daunt him. His was the ascetic, the morally no less than physically athletic, temperament that rejoices in hard, clean striving for its own sake. Such a mind must be up and doing. Its activities will, therefore, naturally issue in research, in bringing truth to light. But Jenkinson was likewise a thinker in whom the purely contemplative mood craved for satisfaction. The precondition of his research must be some vision of the whole.

As it was, the manner of his education was such as to minister conjointly to both sides of his nature, to foster in him the man of science and the philosopher at once and together.

The Oxford School of *Literae Humaniores* is sometimes accused of trying to be all things to all men; and this impeachment is perhaps best met by the plea that such a policy would seem to answer. Here was a man who in his first term of 'Greats' attended biological courses at the Museum, and that with his tutor's blessing. As all roads lead to Rome, so every intellectual avenue might be held to lead to philosophy. And thus it turned out with Jenkinson. He might devote time that he could ill spare to those special problems of biology which were to mean so much for him in after life. He might even be so deeply impressed by the need in that context of empirical methods, of a reasoning resting solidly on observation and experiment, as to be unduly suspicious of the dialectical flights to which philosophy is prone. But a mind so broad and so thorough could not ignore the further issues which the biological theory of development involves. How Becoming in general is related to Being was the question that soon dominated his attention, in those early years when he was still finding himself, still seeking to evolve a sense of cosmic direction.

There was a College society, then in its heyday, which met for purposes of philosophical discussion. Jenkinson was one of the leading spirits in it. He had not yet, it is true, acquired that power of lucid utterance which was afterwards to make him one of the most impressive of University lecturers. But his very earnestness brought him to the front in these youthful debates. In the myriad processes of life which it was his passion to study he divined the workings of law. The organic world was for him no welter of aimlessly competing forms, chance-begun and chance-ended. Somehow there must be determination towards an end, a movement

and growth responsive to a central principle of order; or why the human instinct for truth, why science at all? It was typical of Jenkinson that he never strayed from this path of philosophy, once his feet were firmly planted on it. With him thought and character were so much at one that he had no need to cast about for a leading. Singly-determined as he was in himself towards a life of duty, he saw his world as a counterpart in which every natural process tended towards the ultimate fulfilment of a purpose.

A paper of his exists which well deserves publication if only because it is so much more than a clever arrangement of words, being the expression of a faith by which a man has lived, and died, nobly. It may draw much of its inspiration from Aristotle and Hegel; but is nevertheless original in the fullest sense as proceeding from one who thought for himself —who owned whatever he affirmed. The paper in question first examines the postulates to which the inductive sciences owe their power of advance. In biology no less than in physics strict causation must be assumed. Jenkinson, indeed, is at the level of science so thoroughgoing a determinist that he deprecates the attempt to resort in a biological context to teleological explanations of any kind. Material and efficient causes are the only necessary conditions which science as such has any need to recognize. On the other hand, materialism cannot suffice as a philosophy. 'There is one fact which the materialist forgets to analyse, and which materialism absolutely fails to explain, which is indeed the hardest to understand of all, and that is knowledge; and, with knowledge, those other facts of self-consciousness, feeling, and will.' He concludes, with the idealist, that 'the phenomena which constitute the object of knowledge'—in a word, nature and its laws—'are the creation of the mind itself'. He goes on to urge that 'this mind must be regarded not so much as something outside individual minds, differing from them in capacity, but related to them as they are to each other; but rather as

an inner harmonizing activity between them, of which they are so to speak the expressions, and in which they are included'. In such a faith he finds a common justification for the laborious investigations of the man of science and for the speculative efforts of the philosopher. Herein, too, for both of them, lies the secret of happiness—if they be content to 'strive and hold cheap the strain; learn, nor account the pang; dare, never grudge the throe'. So Jenkinson himself strove and learnt and dared, and no one who knew him can doubt that he lived and died happy.

The time at length came when, his course of the humanities accomplished, he must qualify for that scientific career on which he had set his heart. The omens were by no means propitious. As a schoolboy at Bradfield he had botanized with zeal, some of his finds being recorded in Druce's *Flora of Berkshire*. At Oxford, again, though as Scholar of Exeter he had taken the usual classical schools, he was allowed, as has been mentioned, to attend a few biological classes by way of an intellectual luxury. Such being his entire record as a student of science, he must evidently start from the very bottom of the ladder as regards the special instruction in biology which he now required. Nor can it be said that Oxford makes adequate provision in the way of endowments for those who, having obtained an education in general culture, seek after graduation a technical training in some particular branch of learning or research.

Jenkinson, however, was the last man to be turned aside from an ideal aim by material considerations. Working hard and spending little, he studied zoology for some time at University College, London, under the late Professor Weldon. In the latter he found a kindred spirit—a man whose whole nature found its satisfaction in the pursuit of knowledge. And so he continued, immersed in research, and, as his letters to his friends at Oxford showed, perfectly happy in his chosen vocation, until at length he was appointed to the

teaching staff of the Department of Comparative Anatomy at Oxford. Such junior posts carry with them a mere pittance in the way of remuneration; but, fortunately for the University, there is no lack of young men of first-rate talent ready to serve in the ranks of science on a slender ration. It may even be that hard conditions help to make the pioneer. Certainly, for a man of Jenkinson's strenuous and disinterested temper, the way was clear ahead, once a definite function was assigned him.

Six years after taking his Bachelor's degree he published, as the firstfruits of his researches, a study entitled 'Early Stages of the Development of the Mouse';[1] this being partly the result of work done at Utrecht in the laboratory of the illustrious Dutch zoologist, the late Professor Hubrecht. Five years later he became Doctor of Science, a distinction awarded at Oxford for writings held to display originality in some high degree. Almost from the first his chief interest had lain in embryological questions, and it was appropriate that, in the year after he had received his doctorate, the post should be created for him of University Lecturer in Comparative and Experimental Embryology. A few years later, in 1909, his College, Exeter, on the staff of which he had already been serving as lecturer, was proud to elect him to its sole Research Fellowship, which, tenable as it is for but a limited term of years, can boast a long list of distinguished holders. This year was marked by the publication of his *Experimental Embryology*, the first comprehensive English text-book on the subject, and one that is not likely soon to be superseded. In 1913 another text-book, of no less excellent quality, appeared under the title *Vertebrate Embryology*. So much must suffice as a bare record of the steps by which he was steadily mounting upwards in the scale of usefulness and honour.

Then the war broke out. Jenkinson was now past forty years

[1] *Quart. Journ. Micr. Sci.*, 1900.

of age. He was married, and most happily married, his wife taking a keen and active interest in his scientific pursuits. He was recognized not only in his own University but in the world at large as a foremost authority in regard to his special subject. Yet, though on so many grounds he might have rightfully disregarded the call to arms, he did not hang back for a moment. Soon after the declaration of war he enrolled himself in the Oxfordshire Volunteer Training Corps, and in the following January accepted a commission as Lieutenant in the 12th Battalion of the Worcestershire Regiment, being gazetted Captain three months later. He was one of six officers specially selected for service in the Dardanelles, and left for Gallipoli early in May. Upon landing there he was attached to the 2nd Battalion of the Royal Fusiliers; and, after a little more than a week's experience of the trenches, he took part in the general advance of June 4, and fell in action on that day.

Of such an end one can only say that it was worthy of his life. He had always given the best of himself to some ideal cause, whether it was that of science or of his country. This, indeed, was the very secret of his charm for those who were his personal friends—that he was utterly single-natured, simple and strong, at one with the reason and law which he instinctively discerned in the seeming chaos of nature and human life. Oxford will remember him sadly, yet proudly, as a true son of hers, who, thanks to her ancient kindly discipline of mingled work and play, learnt 'to philosophize without softness'—at once to love wisdom and to play the man.

R. R. Marett.

CHAPTER I

INTRODUCTORY: GROWTH. STRUCTURE OF THE GERM-CELLS

EMBRYOLOGY is the study of development, and when used in its widest sense signifies the inquiring into the reproduction of specific form in the individual organism, by whatever means that reproduction may be brought about, whether by the development of a single cell, of a bud, or by the regeneration of a lost part or parts.

But in a narrower and perhaps commoner sense Embryology is the investigation of the first of these processes, of that series of changes by which there is produced from a single cell a new organism which is like the parents that gave it birth. This cell is usually, though not always, a fertilized egg-cell produced by the union of the two germ-cells, the ovum and the spermatozoon; while these cells therefore are the material basis, the development of the product of their union is the mechanism, of inheritance.

To the inquirer into the phenomena of development two methods are open. Either he may observe and describe the sequence of changes in as many forms of animals as possible—and in that way Comparative Embryology has been built up—or he may, looking upon development as one of the functions of the organism, add to observation experiment with the deliberate intention of discovering the causes of each step and so of the whole process, of establishing general laws expressing the relation between the various antecedents—given in the initial structure of the germ-cells and in the external environment—and their consequents, under which general laws fresh particulars may be subsumed, by which they may be explained, and from which predictions may be made. This is indeed the

aim and scope of Experimental Embryology, and our duty in these lectures will be to discover what progress recent research has made towards the achievement of that ideal.

In development three processes may be discerned, quite distinct from one another but taking place concurrently. These are growth, or increase of size, nuclear and cell-division, and differentiation or, as Herbert Spencer defined it long ago, increase of structure.

A few well-known examples will serve to illustrate this.

The unripe but full-grown ovum of the Sea-urchin (*Strongylocentrotus lividus*) is a minute spherical body, of a faint orange colour due to the presence of a pigment at the surface (Fig. 1). The large nucleus is excentrically placed on that side on which there is a passage—the micropyle—in the external jelly membrane. After maturation, the female pronucleus, much smaller than the original nucleus, lies at first excentrically under the micropyle, into which the polar bodies have been extruded, but later wanders from this position. At the same time the pigment is withdrawn from the larger part of the surface, and concentrated in a superficial zone which is placed—usually though not always—subequatorially, that is parallel to the equator and nearer the vegetative pole. The equator is of course the plane passing through the centre of the egg and at right angles to the axis, while the axis is the line passing through the micropyle and the centre of the egg, the micropyle end of the axis being known as the animal, the opposite as the vegetative pole. We shall have occasion to see later on that this polarity of the ovum depends also upon the intimate structure of the cytoplasm.

The egg is now ready for fertilization. Shortly after fertilization it begins to segment, and the planes of division pass through the egg-substance in a perfectly definite and regular way. The first division is meridional (including the egg-axis), and therefore separates the egg into two equal blastomeres, each of which has a similar share of the large unpigmented region of the animal hemisphere, the pigment-ring, and the smaller unpigmented region around the vegetative pole (provided, of course, the ring has its usual

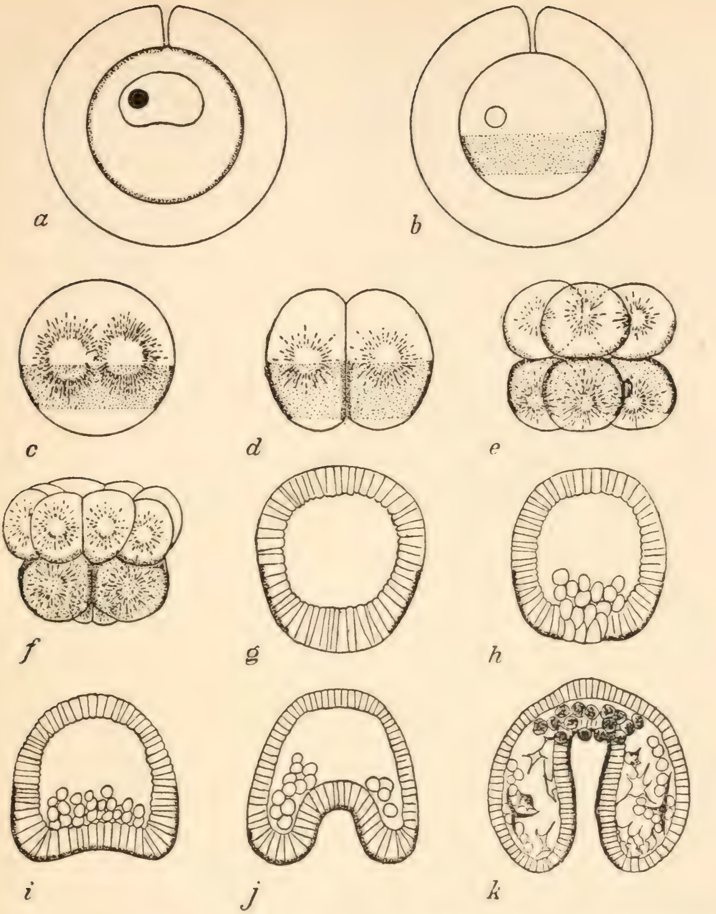


FIG. 1.—Normal development of the sea-urchin *Strongylocentrotus lividus.* (After Boveri, 1901.)

The animal pole is uppermost in all cases, and in the first two figures the jelly with the canal (micropyle) is shown.

a, primary oocyte, the pigment is uniformly peripheral.

b, ovum after extrusion of polar bodies. The pigment now forms a subequatorial band. The nucleus is ex-axial.

c, *d*, first division (meridional).

e, 8 cells, the pigment almost wholly in the vegetative blastomeres.

f, formation of mesomeres (animal cells) by meridional division : the vegetative cells have divided into macromeres and micromeres.

g, blastula. *h*, mesenchyme blastula.

i, *j*, *k*, invagination of the pigmented cells to form the archenteron of the gastrula. In *j* the primary mesenchyme is separated into two groups, in each of which, in *k*, a spicule has been secreted. In *k* the secondary, pigmented mesenchyme is being budded off from the inner end of the archenteron.

subequatorial position). The second divisions, or more properly the two divisions of the second phase, are again meridional, and there are now four blastomeres, each of which has a similar share of the three regions of the egg. But at the next cleavage, which is equatorial, four unpigmented animal cells are separated from four vegetative cells, each of which has one quarter of the pigmented ring and of the smaller unpigmented area.

The direction of the divisions of the fourth phase is different in the animal and in the vegetative hemispheres. In the former it is meridional, producing a ring of eight cells, while in the latter it is latitudinal (parallel to the equator) and unequal, resulting in four large blastomeres (macromeres), which take the pigment, and four small ones, the micromeres, round the vegetative pole.

Division proceeds with regularity and synchrony, at least in some regions of the ovum, for some little time yet, but we need not follow the details. Eventually the divisions become irregular. The final result of segmentation is the blastula, or hollow sphere of cells, disposed in a single layer round the central blastocoel or segmentation cavity. The cells are ciliated, and the blastula escapes from the egg-membrane. In the blastula the same three regions are present as in the unsegmented egg, the large unpigmented, the pigment ring, and the small unpigmented. Segmentation has therefore merely cut up the unlike material of the egg into small pieces, and beyond the segmentation cavity and the cilia there has been formed no new structure, there has been no differentiation.

The next step is the development of the primary mesenchyme. One by one the cells of the small vegetative unpigmented area become amoeboid and migrate into the blastocoel. There they quickly arrange themselves in two groups, of which one lies upon the right, the other upon the left, of the future median plane. Each group secretes a triradiate calcareous spicule, which is the rudiment of the skeleton of the Pluteus larva. The two groups are connected by two curved lines of cells, one of which, on the future

ventral side, consists of a single cell-row, the other, dorsal, of two or more rows. The embryo is now visibly bilateral.

By the immigration of the primary mesenchyme the pigmented cells are naturally brought down to the vegetative pole, and they are now invaginated into the interior. The inner tube so formed is the archenteron, the aperture of invagination the blastopore. The archenteron contains the material for the body cavity and water-vascular system and the alimentary canal, the blastopore persists as the anus. Cells are budded off from the inner end of the archenteron to form the secondary mesenchyme, and then this inner end enlarges, divides into two, and these are nipped off as the right and left coelom-sacs. Each of these later divides into three—pre-oral coelom, hydrocoel, and posterior coelom; but that is a process which we cannot follow here.

The rest of the archenteron is now the gut. It quickly becomes divided into the fore-, mid-, and hind-guts. It is inclined towards one, the ventral, side, where the fore-gut soon unites with an ectodermal depression, the stomodaeum. By perforation at the point of union the mouth is formed.

Meanwhile the ventral or oral side has become flattened and square, the opposite dorsal side convex, and the 'prism' form is attained. A sense-organ—a tuft of long cilia borne by a patch of thickened ectoderm—is developed at the anterior end.

The edges of the square oral side now become thickened, while their cells put out long cilia, so producing the circum-oral ciliated ring, by means of which the larva swims. The four corners of the ring are then pushed out into the four primary arms of the Pluteus, two anterior or antero-lateral, two posterior or postero-lateral, and each is supported by a rod of the skeleton. Of the three radii of the primary triradiate spicule, one grows into the postero-lateral arm on each side, one forms a horizontal bar reaching to the middle line below the gut, and the bar supporting the antero-lateral arm is a branch of this. The third radius passes dorsally to the convex side, and becomes the apical or body rod. Its extremity is swollen and club-shaped. The arms and their

supporting skeletal rods quickly lengthen, and the young Pluteus assumes its 'easel' shape (Fig. 2). Into the later development of additional arms, ciliated epaulettes and so on, and into the formation of the body of the urchin on the left side of the Pluteus, we need not stay now to inquire.

As a second example we shall take another type whose egg has been the subject of frequent and indeed classical experiments, the Frog.

The full-grown but unfertilized egg of the Frog is a spherical

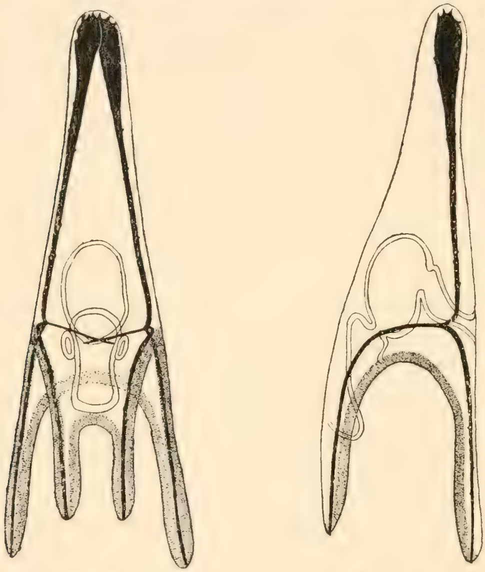


FIG. 2.—Pluteus of *Echinus microtuberculatus* from in front and from the side. (After Boveri, 1896.)

body, with a polarity which depends on the disposition of yolk and cytoplasm, on the arrangement of the pigment, and on the position of the nucleus. The egg is telolecithal; that is, the yolk is so disposed that the yolk-granules on one side are larger and more numerous, on the other side smaller and less numerous, and consequently the cytoplasm more abundant on the latter, less so on the former side; the distinction between the two being, however, not sharply marked but gradual. There is in fact a gradual increase of cytoplasm in passing from one

side to the other, a gradual increase of yolk in passing in the opposite direction. Since the egg is spherical one line, which is different from all others, may be drawn through the centre of the protoplasmic portion at the surface, the centre of the egg itself, and the centre of the yolk portion at the surface. This line is the egg-axis, and its ends or poles are obviously unlike, the protoplasmic pole being known as the animal, the yolk-pole as the vegetative. The equator of the egg is the plane passing through the centre at right angles to the axis. It will be evident that since the cytoplasm and the yolk are symmetrically distributed round the axis, the egg may be divided into precisely similar halves by any plane that includes the axis, but by none other. In other words, the egg is radially symmetrical about its axis.

This radial symmetry is further emphasized by the arrangement of the pigment, which is deposited in the form of minute granules in a superficial layer in the animal portion and extends some little way, to a variable extent in different eggs, below the equator into the vegetative hemisphere. The boundary between pigmented and unpigmented portions is thus a circle parallel to the equator. Lastly, the nucleus—the germinal vesicle in the immature egg, the female pro-nucleus in the mature egg, and the two pro-nuclei in the fertilized egg—lies in the axis, but excentrically, in the animal hemisphere.

As a result of fertilization, however, this primitive radial symmetry is lost, and replaced by a bilateral symmetry, for, opposite the point of entrance of the spermatozoon (which is somewhere in the animal hemisphere), there appears on the border of the pigmented area a grey crescent (Fig. 3), due to the disappearance of the superficial pigment into the interior. The egg is now divisible into similar halves only by a plane including the axis and passing through the middle point of the grey crescent, and about this plane it is now bilaterally symmetrical.

We shall see that this plane of symmetry of the fertilized but unsegmented egg is approximately coincident with the median plane of the embryo, since the side of the grey

crescent becomes dorsal, the opposite side (on which the spermatozoon had entered) ventral, while the anterior end is just above the animal pole, the posterior end therefore just below the vegetative pole.

Segmentation now occurs, the divisions of the first few phases passing through the egg-substance in a perfectly regular and definite way, the first being meridional, the

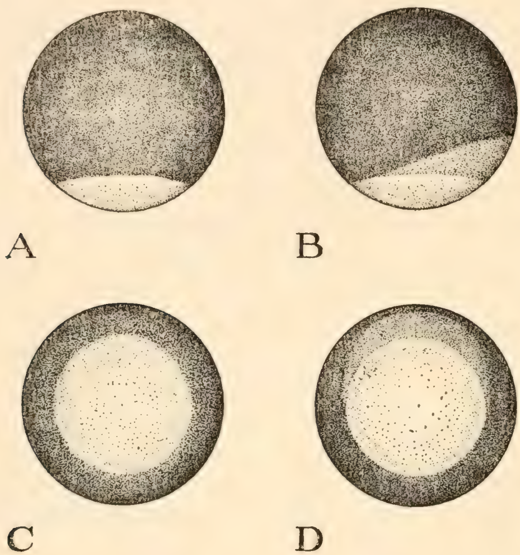


FIG. 3.—Formation of the grey crescent in the frog's egg (*R. temporaria*). A, B from the side; C, D from the vegetative pole. In A, C there is no crescent, in B, D a part of the border of the pigmented area has become grey.

second meridional and at right angles to the first, the third latitudinal, the fourth meridional, the fifth latitudinal. This is the typical sequence, but variations from this radial type are frequent. Subsequent divisions are irregular, tangential divisions occur, and a segmentation cavity is formed. It is noticeable from the very beginning that the animal region of the egg divides more rapidly than the vegetative region, owing to the retarding influence of the yolk. Hence at the end of cleavage the animal hemisphere consists of small cells with

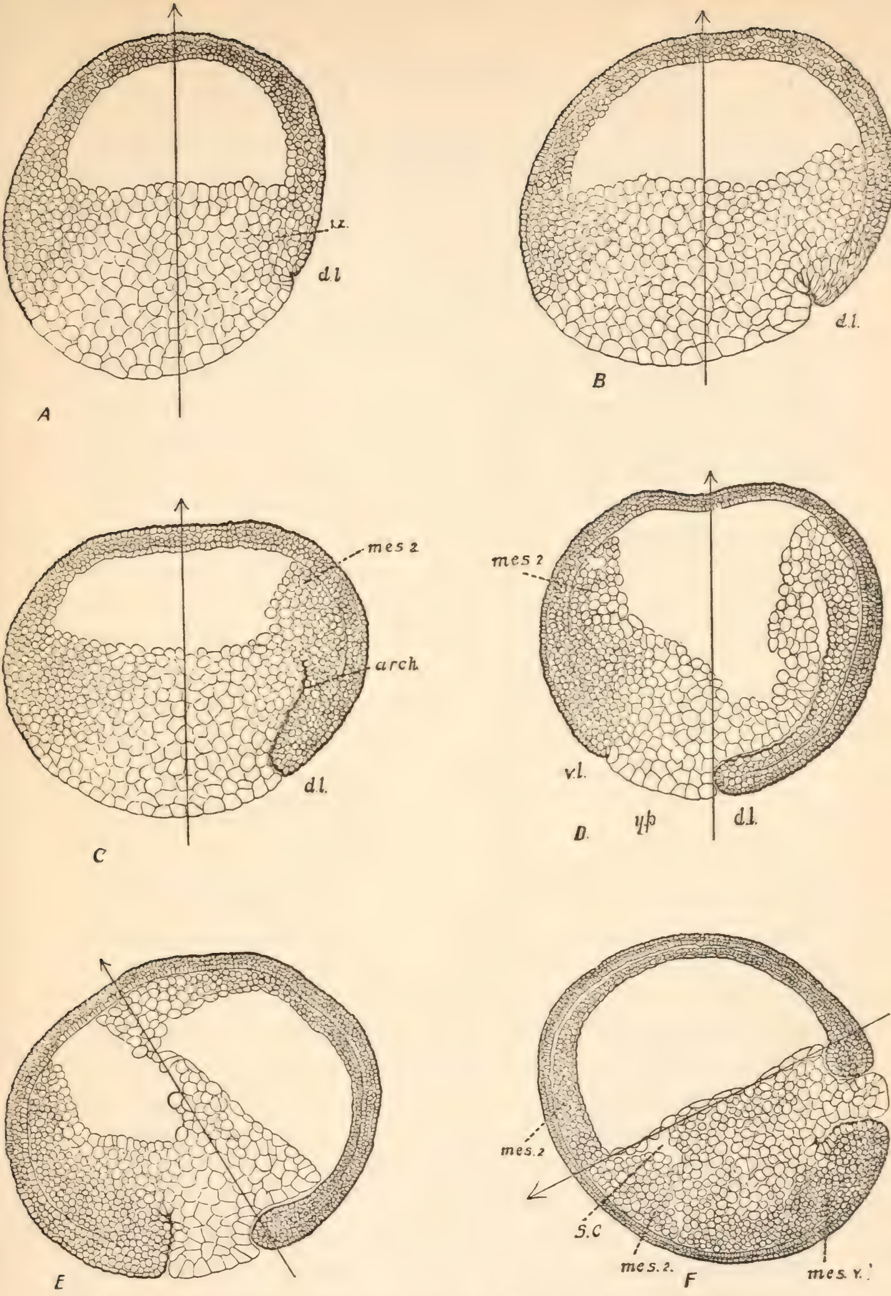


Fig. 4.—Sagittal sections through the frog's egg during the formation and closure of the blastopore. A–D, Before rotation; E, During rotation; F, After rotation.

The arrow marks the egg-axis, its head the animal pole. *d.l.*, dorsal lip; *v.l.*, ventral lip of the blastopore; *s.c.*, segmentation cavity; *arch.*, archenteron; *y.p.*, yolk-plug; *i.z.*, intermediate zone; *mes.v.*, mesoderm formed below the ventral lip; *mes.* 2, mesoderm formed from the yolk-cells pushed into the segmentation cavity.

small yolk-granules disposed in about four layers in the roof of the segmentation cavity, the vegetative hemisphere of large cells with large and abundant yolk-granules in the floor of the segmentation cavity. The distinction between the two regions is not however abrupt, but gradual: about the equator are cells of intermediate size and structure. The superficial animal cells receive the pigment. The segmentation cavity lies in the animal hemisphere. Apart from the formation of this cavity there has been no differentiation during cleavage: the unlike material of the ovum has merely been cut up into unlike pieces, the characters of which in each region depend directly on the structure of that region in the unsegmented egg. But after cleavage comes differentiation, and the first step in this is the formation and closure of the blastopore, during which the material for the germinal layers is laid down, and a new cavity, the archenteron, developed (Fig. 4). The blastopore is formed and closed bilaterally, since the dorsal lip appears first (on the side of the grey crescent, just below the equator), the right and left lateral lips next, the ventral lip last, and since the overgrowth of the fold of pigmented cells is most rapid at the dorsal, least rapid at the ventral, and at an intermediate rate at the lateral lips in between. The archenteron, the cavity developed between the blastoporic fold and the yolk which it covers up, is therefore also most extensive below the dorsal lip, that is, anteriorly. As the yolk-cells are pushed into the segmentation cavity the latter becomes obliterated, while the archenteron is enlarged. From its thin roof are formed the notochord, the dorsal mesoderm, and the roof of the gut; from its thick floor (the yolk-cells), the ventral mesoderm and the floor of the gut.

The blastopore, which has meanwhile been reduced to a small circle, now closes by approximation of its lateral lips, the yolk-plug being withdrawn. The nervous system arises in the form of the pear-shaped medullary plate of thickened ectoderm upon the dorsal side. The medullary folds rise up along the edges of this plate, bounding the medullary groove, which by the coalescence of the folds becomes converted into the closed canal of the central nervous system. Gill-plates

and sense-plates are developed at the sides of the head, a stomodaeum is pushed in in front, a proctodaeum behind, and the head becomes separated by a slight constriction from the neck. The tail grows out above the proctodaeum, the external gills appear, the olfactory pit, eyes, auditory vesicles, heart, gill-slits, coelom, pronephros are developed, and the embryo is ready to hatch out as the tadpole. We need follow the development no farther, but enough has been said to illustrate the occurrence of the three processes of growth, cell- and nuclear division, and differentiation.

The third example will be one of those cases in which it is possible to trace back each organ or system of organs in the body to a particular cell or cell-group in the segmenting ovum, to state in fact its cell-lineage. This is the round worm, *Ascaris megalocephala*. The egg of *Ascaris* is very small and spherical. In it may be distinguished, in addition to the cytoplasm, some yolk globules (specifically lighter than the cytoplasm) massed together on one side: the arrangement is therefore telolecithal and the symmetry similar to that of the frog's egg. There are also some spherules of a clear substance, placed in the animal region.

The first division (Fig. 5) is equatorial, separating an animal cell AB or S_1 from a vegetative cell P_1. When the spindles appear for the second division, it is seen that in the cell S_1 the spindle axis is placed at right angles to the egg-axis, while in P_2 the spindle lies in the egg-axis. S_1 is therefore about to divide meridionally, P_1 latitudinally, and when the division has been accomplished, the cells A and B resulting from the division of S_1 and S_2 or $E\,M\,St$ and P_2 resulting from the division of P_1, are arranged in a characteristic T shape, the cell P_2 being nearest the vegetative pole at the bottom of the stem of the T.

The very remarkable phenomenon of the diminution of the chromosomes can at this stage (metaphase of the mitosis prior to the division) be seen in the cell S_1 or AB. As the four Y-shaped chromosomes lie in the equator of the spindle the middle portion of each becomes transversely divided into a row of small granules, while the ends are swollen. The

whole row then undergoes longitudinal fission, and the daughter rows pass to the opposite centrosomes in the anaphase of the mitosis, to form the daughter nuclei. The swollen ends are however cast out into the cytoplasm, quite irregularly, passing sometimes into both, sometimes into one only of the blastomeres A and B, and disintegrate and disappear. The cell in which the chromosomes are thus diminished is purely somatic; it gives rise to the ectoderm of the embryo. In the other cell, P_1, the chromosomes remain intact. This cell is the parent not merely of certain somatic cells, but also of the future germ-cells, and we shall see that in the course of segmentation each cell which contains in itself the material for the future germ-cells, or lies in the germ-track, retains intact chromosomes, while in every cell that is destined to give rise only to some part of the soma, the chromosomes undergo diminution. The cell $E\,M\,St$ is such a cell; it contains the material for the endoderm, some of the mesoderm, and the stomodaeum, while from its sister cell, P_2, the gonad, as well as some mesoderm, is to be derived.

The P_2 cell now shifts round to one side (the future posterior end), so that the four cells lie in a rhombus. They prepare for the next division. A and B are divided lengthways into right and left moieties, the chromosomes appearing in the 'diminished' form as granules.

$E\,M\,St$ (S_2) divides transversely into an anterior cell, $M\,St$ (mesoderm and stomodaeum), and a posterior cell, E (endoderm). In the mitosis, diminution of the chromosomes occurs. P_2 divides with intact chromosomes into a lower anterior cell, P_3, and an upper posterior cell, C (or S_3). In this cell diminution will occur prior to its division. It gives rise to mesoderm.

By further divisions the number of ectoderm cells is increased (derivatives of AB), and of endoderm cells likewise, the mesoderm (M) is separated from the stomodaeum (St) material, and P_3 divides into P_4 and D (or S_4). The latter is somatic (mesoderm), and diminution of the chromosomes occurs in it. P_4 is now purely germinal, and the chromosomes remain intact in all its descendants.

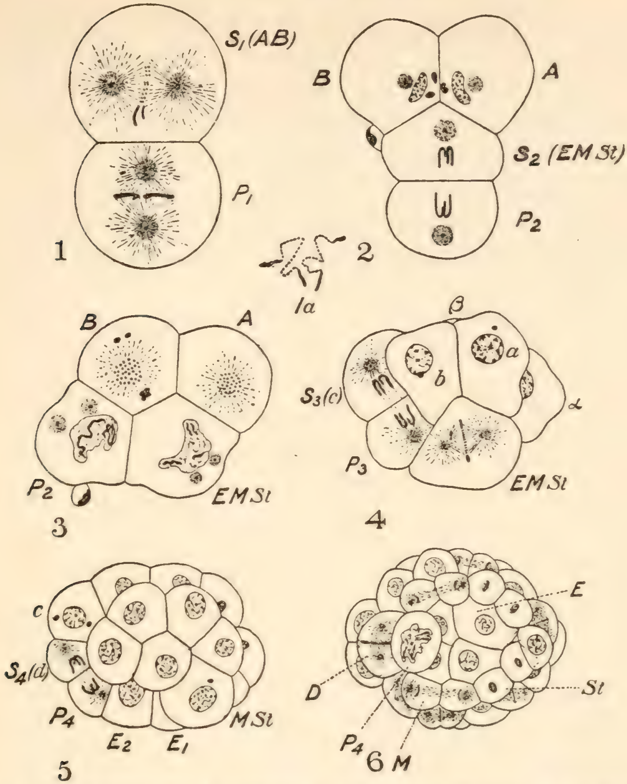


FIG. 5.—The process of chromatin diminution as seen in the somatic cells of *Ascaris megalocephala*. (After Boveri, 1899.)

1. Mitosis in the 2-celled stage. In the first somatic cell (S_1 or AB), the primary ectoderm, the chromatin undergoes diminution, not in the germ-cell (P_1). 1*a*, chromosomes being 'diminished'.

2. 4-cell stage, T-shape. In A and B the discarded masses of chromatin are seen. S_2 ($E\ M\ St$), second somatoblast or endomesostomodaeal rudiment.

3. 4-cell stage. lozenge-shape. In A and B the next mitosis is beginning, in P_2 and $E\ M\ St$ the nuclei are in the resting stage. A is anterior, A and B are dorsal, all four cells lie in one plane, the sagittal plane of the embryo.

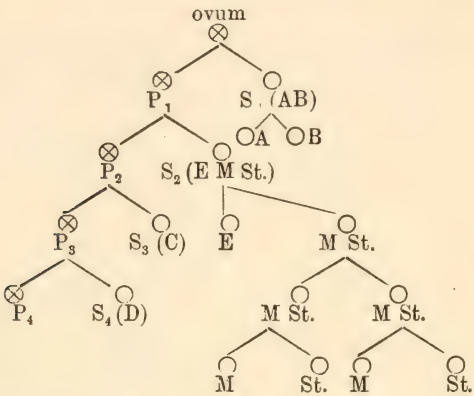
4. In $E\ M\ St$ the chromatin is being diminished. Division of P_2 into P_3 and S_3 (C), the secondary ectoderm. *a*, *b*, primary ectoderm of right, *α*, *β*, primary ectoderm of left side.

5. The endoderm (E_1, E_2) has now been separated from mesoderm and stomodaeum. P_3 has just divided into P_4 and S_4(d, tertiary ectoderm.

6. Diminution of chromatin in S_4 (D). The four endoderm cells (E) beginning to be invaginated: on each side two mesoderm cells (M) in which granular chromosomes may be seen, and two stomodaeal cells (St). Ventral view.

When segmentation is concluded the blastula stage is reached, with a segmentation cavity. Into this pass the mesoderm cells, while the endoderm is invaginated to form the gut. The blastopore is shifted to the anterior end, where it is encircled by the stomodaeal cells.

The cell-lineage which we have just described may be displayed in diagrammatic form thus: the cells in which the chromosomes remain intact being indicated by a cross.



The egg of *Ascaris* has been made the subject of important experiments by Boveri.

And now let us turn to our main thesis, the inquiry into the causes of growth, cell-division, and differentiation.

We shall begin with growth.

Growth is increase in size, and may be measured by increase in weight, sometimes by increase in other dimensions, such as stature, girth, and so on. Growth is partly the increase of living material, in part the increase of substances secreted by that living material. These secretions may be intra-cellular, for instance the contractile substance of a muscle fibre, or extra-cellular, as the chitinous cuticle of an Arthropod; they may be organic, as in the examples quoted, or inorganic, such as sponge spicules or the calcareous matrix of bone. Growth obviously depends upon the assimilation of food-material, which in early stages is usually provided by the yolk. It

also depends upon the absorption of water, as Davenport's investigations upon tadpoles have shown. The percentage of water in a tadpole's body, for example, increases during the first fortnight after hatching from 50 % to over 80 %: after that the water percentage decreases slightly.

The same high proportion of water in the tissues in early stages, followed later by a decline, has been demonstrated for other embryos, for example the human embryo, as may be gathered from the sixth column in the accompanying table (Table I). At the present moment, however, the main interest in growth undoubtedly centres in the way in which the rate of growth alters during the progress of development.

There is some difference of opinion as to the method by which this rate should be measured.

The table gives the actual weight (x) at certain times, the increments from time to time (Δx), the average increments per unit of time for each of these intervals $\left(\frac{\Delta x}{\Delta t}\right)$, and the percentage increments, or these increments expressed as a percentage of the weight at the beginning of the interval $\left(\frac{\Delta x}{\Delta t} \times \frac{100}{x}\right)$. It is this last magnitude which Minot has proposed to employ as the measure of growth-rate, and if the figures in the fifth column of the table be examined it will be seen at once that the rate is high initially, diminishes abruptly at first, thereafter more slowly to a final minimal value.

The same abrupt descent from an initial high value, followed by a more gradual decline, can be seen in many other cases; for example, in the figures for the change in the post-natal growth-rate of man (Table II). There is a slight but temporary rise about the time of puberty. The graph constructed from the figures resembles therefore a logarithmic graph or curve.

The parts of the body obey the same law as the whole, although of course all the parts are not growing at the same rate. The latter may be illustrated by the changes undergone by the index-value of the parts (the dimension of the part expressed as a percentage of that of the whole) during the course of development.

TABLE I. HUMAN EMBRYO. (FEHLING.)

Age in weeks.	Weight in grammes (x).	Increment (Δx).	Weekly increment $\left(\frac{\Delta x}{\Delta t}\right)$.	Weekly % increment $\left(\frac{\Delta x}{\Delta t} \times \frac{100}{x}\right)$.	% of water.
6	0·975				97·5
17	36·5	35·525	3·23	331·2	91·8
22	100·0	63·5	12·7	34·8	92·0
24	242·0	142·0	71·0	71·0	89·9
26	569·0	327·0	163·5	67·6	86·4
30	924·0	355·0	88·75	15·6	83·7
39	1640·0	716·0	79·56	8·6	74·2

TABLE II. WEIGHT OF MALE BELGIANS IN KILOGRAMS. (QUETELET.)

Time in years.	Weight (x).	Increment (Δx).	Increment pertime $\left(\frac{\Delta x}{\Delta t}\right)$.	% increment $\left(\frac{\Delta x}{\Delta t} \times \frac{100}{x}\right)$.
Birth	3·1			
1	9·0	5·9	5·9	190·3
2	11·0	2·0	2·0	22·2
3	12·5	1·5	1·5	13·6
4	14·0	1·5	1·5	12·0
5	15·9	1·9	1·9	13·6
6	17·8	1·9	1·9	11·9
7	19·7	1·9	1·9	10·7
8	21·6	1·9	1·9	9·6
9	23·5	1·9	1·9	8·8
10	25·2	1·7	1·7	7·2
11	27·0	1·8	1·8	7·1
12	29·0	2·0	2·0	7·4
13	33·1	4·1	4·1	14·1
14	37·1	4·0	4·0	12·1
15	41·2	4·1	4·1	11·1
16	45·4	4·2	4·2	10·2
17	49·7	4·3	4·3	9·5
18	53·9	4·2	4·2	8·5
19	57·6	3·7	3·7	6·9
20	59·5	1·9	1·9	3·3
21	61·2	1·7	1·7	2·9
22	62·9	1·7	1·7	2·8
23	64·5	1·6	1·6	2·5
25	66·2	1·7	0·85	1·2
27	65·9	−0·3	−0·15	−0·2
30	66·1	0·2	−0·07	0·1

It has however been proposed by Robertson that the average increment for a short interval of time, namely, the ratio $\frac{\Delta x}{\Delta t}$, would be the more proper measure of the rate, the velocity of a chemical or physical reaction being indeed expressed in this way.

Now if the graph of the rate as so measured be constructed it will evidently be of a totally different character, rising from a minimum to a maximum, and then descending to a minimum again. In the human being, as a matter of fact, there are four periods in each of which a maximum rate is thus attained: the first before birth, the second at the time of birth, the third about the sixth year, and the fourth about the sixteenth.

Now such a graph resembles that constructed for the rate of an autocatalytic unimolecular chemical reaction, as Robertson has pointed out. Robertson, indeed, following Loeb, suggests that the growth of the body depends on the synthesis of nucleins, and that this involves reactions of this kind. But however that may be, it is not difficult to put to the test the supposed resemblance of the change in the animal growth-rate to the change in the rate of the kind of chemical reaction alluded to. The velocity of such a reaction at any moment is given by the equation

$$\frac{dx}{dt} = kx\,(A-x),$$

where x is the amount of change that has been accomplished at that moment (t), A the final amount to be accomplished, and $A-x$ consequently the amount that still remains to be accomplished at the moment in question.

It follows from this that where $x = \frac{A}{2}$, that is, when the reaction is half over, the velocity attains a maximum, since

$$\frac{d\,\frac{dx}{dt}}{dx} = k\,(A-2x),$$

which is equal to zero when $x = \frac{A}{2}$, and since

$$\frac{dk(A-2x)}{dx} = -2k, \text{ that is, is negative.}$$

Hence, if we suppose the growth-rate of the animal organism to depend in the same way upon x, the amount of growth already accomplished, and upon $A-x$, the amount still to be accomplished at any particular moment, then the theory may readily be checked by ascertaining whether the rate is indeed at a maximum when the growth is half done.

Robertson, calculating from the data given for human and other growth, has found that this is very approximately true. One instance will suffice here, the growth of the human being during the fourth period (second post-natal) from the ninth to the thirtieth year.

If a graph be constructed whose ordinates are the successive rates $\left(\frac{\Delta x}{\Delta t}\right)$, and whose abscissae are the successive weights (x), it will be found that the maximum rate occurs when half the increase of weight to be achieved during this period has been attained. From the formula the theoretical rates for the successive weights may be calculated, and their graph compared with that of the observed rates.

There are means by which the value of the hypothesis may be tried. The value of the constant k for instance may be found, the theoretical weights (x) then calculated and compared with the actual ones. Robertson has found the agreement to be very fairly good.

A botanical instance may be quoted here. The measurements made, many years ago, by Errera for the growth of the sporangium-bearing hypha of the fungus *Phycomyces* show very clearly that the rate ascends to a maximum when the growth is half completed.

The body of a Metazoon is often compared to the sum-total of all the individuals produced by the repeated division of a single Protozoon, and it might perhaps be thought that the rate of growth of this total would alter, in the same way as the growth-rate of a Metazoon. But this is apparently not the case. We know indeed from the researches of Calkins, Woodruff, and others that the rate of division of Protozoa

(Infusoria) changes, periods of rapid division alternating with periods of depression, or slow division, and it would seem from the figures given by Woodruff for the mean rate of division for a succession of five-day periods, that at any moment the rate is roughly proportional to the number of divisions that have taken place since the last period of depression, and to the number that will occur before the next period of depression is reached: also that the rate is at a maximum when half the divisions have occurred. In other words, the rate of division of the Protozoon changes in the same way as the rate of growth of the Metazoon. It follows, of course, that the rate of growth of the former, as expressed by the average increment of the total weight (or other dimension) of all the individuals produced by division of one, cannot obey the same law. At the same time, some figures published by Popoff for the growth of a single individual of an Infusorian (*Frontonia*) between two divisions, indicate that the growth-rate rises to a maximum in the middle of the process, and may therefore possibly depend at any moment on the amount grown already, and the amount still to be grown. If this should prove after further investigation to be really the case, then the growth of the individual Protozoon would be comparable with the growth of the individual Metazoon; in both cases the rate at any moment would depend on the two factors named. A further problem remains. While we can understand that the rate should depend upon the amount of living and growing substance, x, it is not so easy to see what meaning is to be attached to the other factor, $A - x$. In the case of the chemical reaction this is the amount of substance left unchanged. Robertson has suggested that in the organism this is cytoplasm, or some ingredient in the cytoplasm from which nuclein can be synthesized, the synthesis of nuclein being the supposed autocatalytic unimolecular reaction upon which growth depends. The idea is based upon Loeb's assumption that there is such a synthesis during segmentation in the (Sea-urchin) ovum. This is, however, erroneous, since Masing has shown that there is as much nucleic acid in the unsegmented as in the fully segmented egg. In the former it lies in the cytoplasm, into

which it had passed from the nucleus, as 'yolk-nucleus', during the growth of the oocyte; in the latter it lies in the nuclei, which apparently have taken it up. Still, there may be synthesis of nucleins in later stages, and the idea may be substantially correct.

Again, the internal factor A, which gives the definite limit to which the growth can proceed, might possibly be looked for in the capacity of the fertilized ovum to divide a given number of times and no more. If this were so, then the rate of cell-division in the Metazoa would not be governed by the same law as in the Protozoa, if in the latter the rate of division is given by $km(n-m)$, where m is the number of divisions that have taken place at any moment, n the total number that can take place, while in the former the rate of growth is given by $y\,2^m\,(2^n-2^m)$, where y is the initial size of the plasma of the ovum.

More probably it will be necessary to look for a wider formula, which will include both these, as for instance that the processes of cell-division, of synthesis of nucleins, and of synthesis of all those cytoplasmic substances and secretions on which growth depends, are all conditioned by the presence of certain extra-cellular ferments of a certain intensity, whose activities become progressively diminished by the combination of the ferments with the products of the reaction, the reversibility of the reaction, and other causes.

Before bringing this part of the subject to a conclusion it may be pointed out that Minot's expression for the growth-rate (the percentage increment) states the facts in another way, though it fails in not calling attention to both the factors involved. The equation $\frac{dx}{dt} = kx\,(A-x)$

may be approximately written

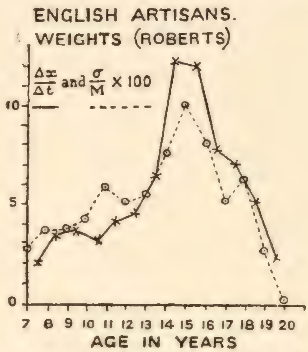
$$\frac{\Delta x}{\Delta t} = kx\,(A-x) \qquad \text{or} \qquad \frac{\Delta x}{\Delta t} \times \frac{1}{x} = k\,(A-x).$$

The expression on the left is proportional to Minot's percentage increment, that on the right the velocity of a unimolecular reaction, the graph for which (logarithmic) resembles the graph of rates constructed by Minot's method.

The next feature of interest presented by growth is the

relation between the growth-rate on the one hand, and the variability of the organism and the degree of correlation between its various parts on the other. With regard to the first, it has been found by various observers that as the growth-rate rises and falls so does the magnitude of the variability (which we may measure by the standard deviation (σ), or better by the coefficient of variability $\left(\frac{\sigma}{M} \times 100, M \text{ being the mean}\right)$. A few instances will suffice.

The way in which the variability goes up and down with the growth-rate will be seen at once from the accompanying graph of the alteration of these quantities during the last growth-cycle of the human being. The data have been taken from the measurements of Roberts for the weights of English artisans.



Again, the same general agreement between these two magnitudes is found in the Trout during the short period, the first ten weeks after hatching, for which data are available, and this is true not only for the growth and variability of the whole body (total length) but for those of the parts as well, eye diameter for instance, length of head and length of caudal fin. The graphs do not indeed run parallel throughout their course, but the fall or rise in variability is accompanied by a fall or rise in the growth-rate. The agreement is perhaps as close as could be expected when it is remembered that a very small portion of the total life of the animal has been under observation, and that this small portion possibly includes one of the points of transition from one growth-cycle to another (see the graphs of the growth-rate of the total length in appendix).[1]

[1] The agreement between growth-rate and variability is much improved by taking the mean increment and not the percentage increment as the measure of the former. I mention this as I was much puzzled by finding that a rise of variability at the end of the period was not accompanied by a rise in the growth-rate when the latter was expressed, as I now believe erroneously, by the percentage increment.

Secondly, it seems that there is a similar relation between growth-rate and correlation, as Boas has pointed out in the case of the human being.

The accompanying table (Table III), which gives the value of the correlation coefficient between several pairs of organs in the young Trout, shows that in many cases—total length and breadth of caudal fin, total length and length of anterior dorsal fin, total length and length of head, head length and eye diameter—there is a significant diminution in the value during the time that shows a decrease in growth-rate. The increases seen in certain cases in the table are within the limits of error.

TABLE III. TROUT LARVAE. CORRELATION COEFFICIENTS (ρ).

Weeks after hatching.	Total length and Breadth caud. fin.	Total length and Length ant. dors. fin.	Total length and Length post. dors. fin.	Total length and Length head.
2	$0·89 \pm 0·01$	$0·76 \pm 0·02$	$0·50 \pm 0·05$	$0·95 \pm 0·00$
10	$0·73 \pm 0·03$	$0·60 \pm 0·03$	$0·52 \pm 0·04$	$0·85 \pm 0·01$

Weeks after hatching.	Head length and eye diameter.	Length ant. dors. and Length post. dors. fin.	Length ventral and Length post. dors fin.	Position of pectoral and of pelvic fin.
2	$0·94 \pm 0·01$	$0·43 \pm 0·06$	$0·52 \pm 0·05$	$0·87 \pm 0·01$
10	$0·65 \pm 0·03$	$0·39 \pm 0·05$	$0·58 \pm 0·04$	$0·83 \pm 0·02$

Boas has brought forward mathematical proof that the magnitudes of the variability and of the correlation coefficient are necessary consequences of those of the growth-rate, rising and falling with it.

But apart from this, considerable interest attaches to this question of the change in the degree of correlation during

development, for high correlation probably points to dependence in development of one part upon another, a factor we shall see to be of the greatest importance, and what throws light upon one may help to explain the other.

The same may be said of the change in variability, for a knowledge of what brings about this change is a step towards the knowledge of the causes of the phenomenon.

We turn next to the second process in development, the division of the ovum, preceded by the karyokinetic division of the nucleus. A discussion of the problems presented by these phenomena requires, however, a brief preliminary account of the structure of the germ-cells, and of their union in the act of fertilization.

The germ-cells—the ovum and the spermatozoon—though very dissimilar in structure, except in one respect, are the products of a history which is almost identical in the two sexes. In this history there are three periods, of multiplication, of rest and growth, and of maturation. In the first period the young germ-cells derived from the primordial germ-cells divide many times, showing at each division of the nucleus the same number of chromosomes as is seen in the tissue-cells. This number, since it is usually even, we shall speak of as $2n$. These cells are spoken of as oogonia and spermogonia respectively.

After a while the divisions come to an end and each cell grows into a primary oocyte or a primary spermocyte as the case may be. It is at this point that the difference between the two sexes becomes manifest.

In the male the growth is not great, the nucleus passes through the prophases of the first maturation division—leptotene, synaptene, pachytene, and formation of heterotypic chromosomes—and then immediately proceeds to the two actual divisions of the third or maturation period. As is well known, the number of chromosomes is reduced from the somatic number ($2n$) to the germ number (n), and very possibly this occurs, as many hold, by a separation of the dissimilar halves of the n bivalent heterotypic chromosomes in the first division,

while the single chromosomes, thus reduced in number, are longitudinally split in the ordinary manner in the second (homoeotypic) division. But however that may be, the number is reduced and each secondary spermocyte (produced in the first division), and again each spermatid (formed from the division of these), contains in its nucleus only one-half of the somatic number of chromosomes. Each spermatid is then metamorphosed into a spermatozoon. This process shows a remarkable similarity in the many forms in which it has been studied: we may take a flagellate vertebrate spermatozoon —that of a Salamander—as fairly typical (Fig. 6). The centrosome of the spermatid divides into two, and these leave the sphere of attraction. The two centrosomes place themselves radially, while the sphere moves round to the opposite (or anterior as it will be) end of the cell. Here it becomes altered to form the acrosome or perforatorium. Of the two centrosomes the inner or anterior places itself close to the nucleus. in the hinder wall of which it becomes embedded. Here it enlarges and elongates to form the so-called middle-piece or neck. The posterior centrosome, from which the axial filament of the tail has in the meantime grown out, becomes transformed into a ring. The ring is eventually broken in two, one half remaining attached to the anterior centrosome, the other travelling a little way down one (the ventral) side of the tail. On the other (dorsal) side of the tail, the fin grows out along the axial filament. The nucleus elongates, and becomes finally dense and very chromatic.

In the female, on the other hand, the growth in the second period is very much greater, since it is at this time that the yolk granules are deposited in the cytoplasm. The nucleus, which has previously passed through the prophases of the first maturation division, makes certain contributions to the cytoplasm; that is to say, substances, which may be solid or liquid, chromatic or achromatic, and are generally spoken of as 'yolk-nucleus', pass from the nucleus into the cytoplasm and are there concerned in the metabolism of yolk-secretion. When growth has come to an end, the enlarged nucleus or germinal vesicle breaks down and its contents are cast into the

cytoplasm, of which they form henceforward a definite and integral part, of great importance, as we shall see, in the early differentiation of the embryo. The spindle of the first matura-

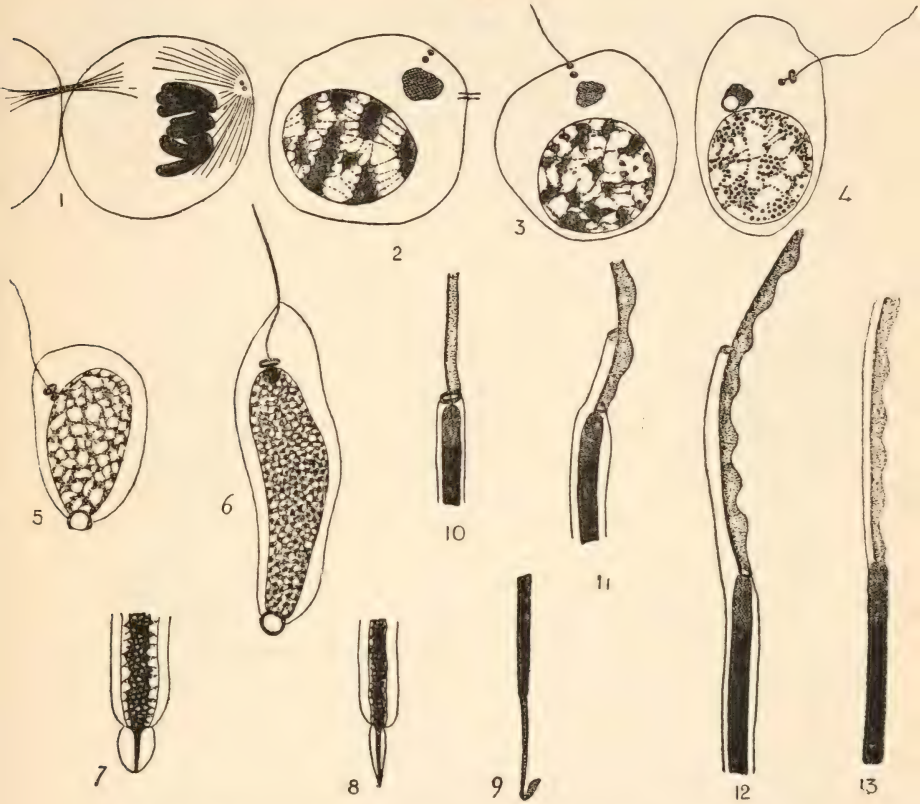


FIG. 6.—Metamorphosis of the spermatid into the spermatozoon in the Salamander (after Meves). 1–6, the whole cell; 7–9, the anterior end; 10–13, the posterior end of the head.

tion division and the heterotypic chromosomes, which are derived from only a very small part of the contents of the nucleus, go to the surface and the two maturation divisions occur. Whereas in the male these divisions are equal, in the female they are very unequal. By the first the first polar body, by the second (which is homoeotypic) the second polar body,

is extruded from the ovum (Fig. 7). The polar bodies are of course minute compared to the egg-cell. The first often divides into two. Thus four cells are produced as in the male, but one

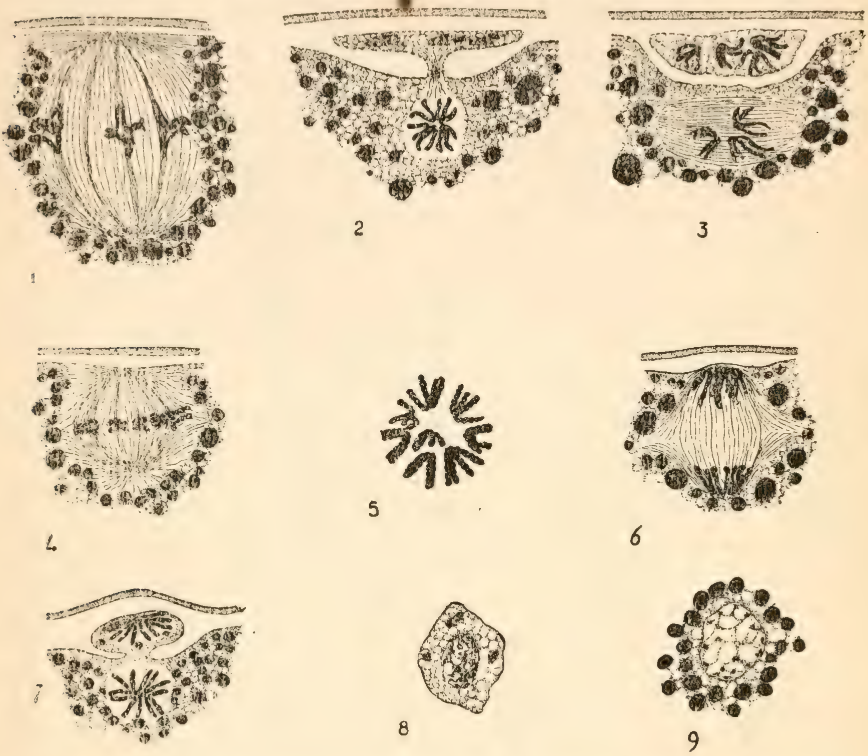


FIG. 7.—The maturation divisions in the female (Axolotl). 1, First polar spindle with heterotypic chromosomes; 2, Extrusion of first polar body; 3, Appearance of second polar spindle; longitudinal division of chromosomes in egg and in first polar body; 4, Second polar spindle radial; homoeotypic chromosomes on equator (metaphase); 5, Polar view of the same; 6, Anaphase; 7, Extrusion of second polar body; 8, Second polar body with resting nucleus; 9, Female pronucleus in resting condition, closely surrounded by yolk-granules.

is large, the actual ovum, the others small (potential ova). Each of these four contains, as does each spermatid, only one-half the ordinary number of chromosomes.

The ripe egg-cell possesses a definite structure, and frequently this structure is polar, as in the case we have been describing,

where the axis with unlike poles (animal and vegetative) is determined by the elongation of the ovarian egg, the formation of the micropyle, and liberation of the contents of the germinal vesicle into the cytoplasm. All telolecithal eggs (e. g. Vertebrate eggs) have a similar polar structure similarly determined by the disposition of yolk, and sometimes by the presence (Frog) of pigment as well, and by the position of the nucleus. Such an egg is divisible into similar halves by any plane which includes the axis. But the egg may be centrolecithal (most Coelenterates), and here the axis is only determinable by the excentricity of the nucleus, though in one interesting case (*Carmarina*) there is an excentric jelly-plasm near the vegetative pole. In some cases (Cephalopods, Insects) the egg is bilateral.

When the germ-cells meet and unite in the act of fertilization the full number of chromosomes ($2n$) is of course restored, but that is not the only nor even the chief event involved in the process. In fertilization four distinct events occur, and sometimes a fifth. The first of these is the extrusion by the ovum of a fluid, the perivitelline fluid; the second is the entrance of the spermatozoon; the third is the appearance of the definitive centrosome and its division into two to form the cleavage apparatus of asters and spindle; the fourth is the union of the male and female pronuclei. To these must be added, in some cases at least, a fifth, the alteration of the structure and symmetry of the egg.

1. The moment a spermatozoon comes in contact (by its acrosome) with the surface of the egg the latter extrudes a fluid, termed perivitelline. This process is often accompanied by the formation of a membrane, the fertilization membrane, as in the Sea-urchin, which prevents the entrance of more spermatozoa. This membrane, in the Sea-urchin, is not preformed, but is to be regarded as the surface-layer of the egg itself, gradually lifted up and separated from the egg by the collection beneath it of a fluid—the perivitelline fluid—which is hypertonic to sea-water. The membrane so formed quickly becomes spherical.

In the Polychaet worm (*Nereis*) the mechanism is rather

different. Here the membrane is preformed—the vitelline membrane. Immediately below it there is a peripheral layer of radial alveoli filled with a gelatinous material. Internal to this is the cytoplasm with yolk-spherules and oil globules, and of course the nucleus. Immediately upon contact of a spermatozoon with the surface of the vitelline membrane the gelatinous material of the peripheral alveoli is extruded as a broad zone of jelly. Sea-water enters and occupies the alveoli, whose walls remain as strands or lamellae crossing the perivitelline space between the membrane and the ovum. The perivitelline space is therefore, as in the Sea-urchin, in a sense a part of the ovum.

In the Frog, a fluid is excreted, and this pushes away from the ovum the vitelline membrane, which remains adherent to the jelly. The ovum is now free to turn in the fluid, and does so until the axis assumes a vertical position with the heavy white pole below. This appears about half an hour after insemination.

2. As soon as the acrosome of the spermatozoon has perforated the surface-layer of the egg-cytoplasm there begins to collect around it a substance, hyaline in appearance (in the Axolotl) and apparently of a more watery consistence than the rest of the cytoplasm. It increases quickly in amount and assumes the form of a cone, the apex of which is directed towards the interior of the egg. It is known as the entrance funnel (Fig. 8). Its base usually projects slightly from the surface: this is the entrance cone (erroneously termed cone of attraction). The entrance-funnel, still increasing, moves on into the interior of the ovum, and the spermatozoon is carried impassively with it. In the Axolotl (and others) the tail is also carried in, but in some cases (*Mus, Nereis,* for instance) it remains outside. Even when taken into the egg it degenerates: it has no part to play in the later stages of fertilization. The acrosome now seems to get caught in the side of the entrance-funnel and, the inward streaming movement of the latter continuing, the head of the spermatozoon is driven on and rotated through 180°, so that the neck (containing the centrosome) now lies at the inner end of the entrance-funnel, while

the head and tail are both directed to the outside. The entrance is now completed. It is effected by the formation, under the influence of the acrosome, of a substance which, by virtue apparently of its capillary properties, moves into the egg and carries the sperm bodily with it. The sperm is in all cases rotated through 180° in the process, so that the middle-piece is inwardly directed.

The sides of the entrance-funnel are lined, in the Axolotl and Frog egg, by pigment dragged in from the surface. This pigment remains as a streak marking the position of the funnel when the latter has disappeared. The entrance-funnel is known in these eggs as the first part of the sperm-path.

A clear, yolk-free area is now developed round the middle-piece or centrosome: this is the sperm-sphere, and from its periphery radiations soon arise and pass out between the yolk-granules, the sperm-aster. The formation of the sperm-sphere is probably also due to the withdrawal of water from the cytoplasm by the centrosome. The centrosome is in any case used up during the process, and entirely disappears. The sperm-head, now detached from the tail, quickly shortens and thickens to become the sperm-nucleus, or male pronucleus.

3. The definitive centrosome is now developed. It arises in the Axolotl from the sperm-nucleus. The nuclear membrane opens on the inside, and there emerges a dense rounded body, or more probably there emerges something (? nucleic acid) which produces this body by precipitation of the proteins of the egg. However formed, this body is the definitive centrosome. The sperm-nucleus is now moving along what is known as the second part of its path to meet the female pronucleus. The latter has moved from its position at the animal pole towards the centre of the egg, usually but not necessarily along the egg-axis. Thus the second part of the path may or may not lie in the same meridional plane as the first part (entrance-funnel), and in the former case it usually makes an angle with the first part, since it is directed to a point approximately in the axis and at a fixed distance from the animal pole, while the point of entrance of the spermatozoon may be anywhere in the animal hemisphere (see below).

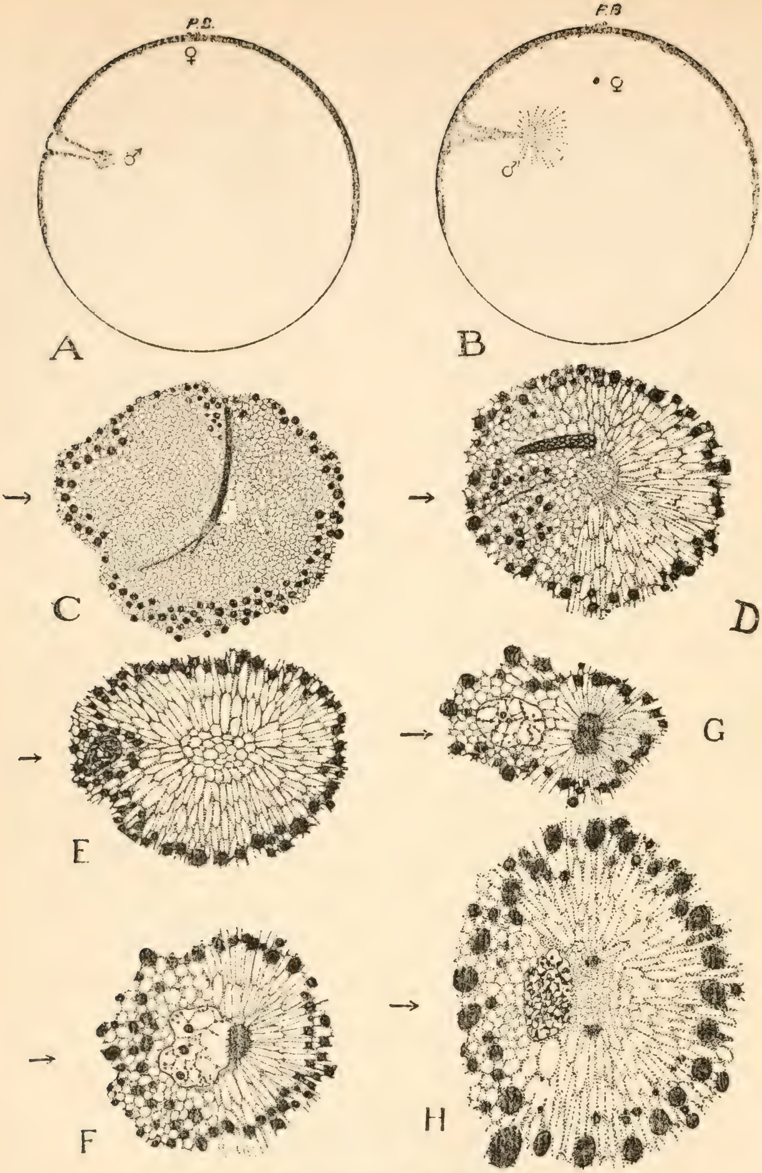


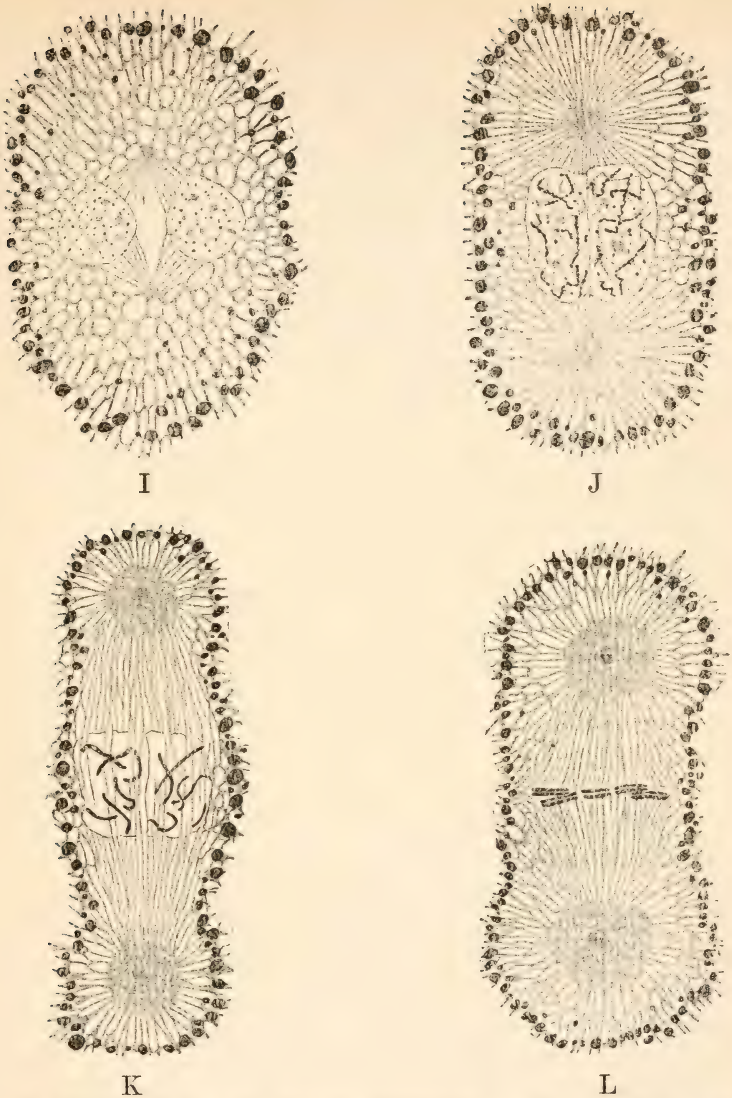
Fig. 8.—Fertilization in the Axolotl.

A and B, Meridional sections of the whole egg. A, Formation of entrance-funnel (first part of sperm-path). B, Formation of sperm-sphere and aster; ♂ male pronucleus; ♀ female pronucleus; *p.b.*, the two polar bodies.

C, Formation of the sperm-sphere round the middle piece (anterior centrosome); parts only of the head (black) and tail are shown.

D, Formation of the sperm-aster. The centrosome has disappeared; the head, beginning to be vacuolated, is separated from the tail.

E, Further shortening and vacuolation of the sperm-nucleus. There is still no centrosome.



F, Appearance of the definite centrosome. G, H, Division of the centrosome.

(In C–H the arrow marks the direction of entrance of the spermatozoon.)

I, Approach of the two pronuclei. Formation of spindle-fibres.

J, Formation of asters, elongation of spindle, further enlargement of pronuclei, and appearance of chromosomes.

K, Further elongation of spindle, and formation of a centrosphere round each centrosome. The pronuclear membranes are breaking down and the spindle-fibres passing in.

L, The fully-formed fertilization spindle. In the equator are the chromosomes, now longitudinally split, and attached to large spindle-fibres. In each centrosome the centriole has divided.

The definitive centrosome now divides at right angles to this second part of the path, and in a plane parallel to the equator of the egg. Preceded by its two centrosomes and the sperm-aster, the sperm-nucleus continues to move towards the female pronucleus. When they meet, the line joining them, that is the second part of the sperm-path, is naturally at right angles to the line uniting the two centrosomes (Fig. 8).

The ovum itself has no centrosome, and it is an invariable rule for the female centrosome, even though present in the polar divisions, to disappear. There is also little doubt that in all cases the definitive centrosome is of male origin, though whether derived from the centrosome present in the middle piece, or developed *de novo*, as in the Axolotl, is not certain. It may be said, however, that while there is very little evidence for the continued persistence of the original centrosome in any case, a conclusive demonstration has been given recently by Lillie of the origin of the cleavage centrosome from the sperm-nucleus, in *Nereis*. For in *Nereis*, first the middle piece is normally left outside with the tail, but a centrosome nevertheless is seen later in the sperm-aster; and secondly Lillie has shown by a very beautiful experiment that any fragment of sperm-head introduced into the egg rotates, and develops at its inner end an aster and a centrosome. The experiment consists in centrifuging the egg during the entrance of the sperm. The jelly, being lighter than the egg itself, is dragged off towards the centripetal end of the tube, and removes with itself any part of the spermatozoon that may still be outside the egg. It is possible in this way to remove the hind end of the head but allow the front end to go in. The part that enters behaves as described. Sometimes it is by the violence of the operations divided into two: each piece then rotates, and forms aster and centrosome.

With the evidence before us it would hardly be too rash to suppose that it is a general rule for the definitive centrosome to be not only a sperm-centrosome, but one developed *de novo* from the sperm-nucleus.

4. The pronuclei now lie side by side between the two centrosomes. From the latter spindle-fibres grow out and

impinge upon the nuclear membranes, while in other directions similar outgrowths constitute the asters. The spindle quickly elongates while its fibres break through the nuclear membranes and pass continuously from pole to pole. The chromosomes are formed independently in each nucleus, and the two sets, paternal and maternal, each to the number of n, lie side by side in the equator. There they are divided lengthways in the usual fashion and their halves pass to the spindle-poles. The nucleus of each of the first two blastomeres and eventually of each cell in the body thus receives a full set of chromosomes from each parent.

It is not unnatural that the union of the two pronuclei should have led the discoverers of the fact to regard it as being the essence of fertilization. The opinion was further supported by the phenomena of conjugation in certain Infusoria, where apparently, there is merely an exchange of micronuclear material between the gametes, and by the similarity of the two germ-cells in respect of their nuclei, the latter being conceived as the sole vehicles for the transmission of the inheritable characters of the species, a task believed to be performed equally by the two sexes.

Now whatever views we may come to hold as to the rôle of the nucleus in inheritance, it is assuredly not true that both nuclei are necessary for the production of a normal individual. For in the first place in parthenogenesis, natural and artificial, only the female nucleus is present, and yet a normal embryo or larva is developed and reared.

In the second place in what is called merogony, the enucleate fragment of an ovum may be fertilized and give rise to a normal larva.

We are obliged therefore to look for the meaning of fertilization elsewhere, and we find it in the restoration of the lost power of nuclear and cell-division. The ripe germ-cells are cells which have come to the end of their power of reproduction by division: in the act of fertilization that power is restored. It is mutually restored, for in ordinary fertilization we see the egg-cell with both sets of chromosomes divide

as a result of this stimulus, while in merogony we see the paternal chromosomes alone so stimulated. Nor is the mechanism of the process far to seek. The male cell introduces or manufactures a centrosome, the female cell provides the cytoplasm wherein the centrosome can make the necessary division-apparatus. The two cells are thus mutually complementary. The experiments we shall have to describe in the next lecture suggest that the stimulus so conveyed to the egg by the spermatozoon may be of a physical or chemical nature.

5. Finally in some cases the spermatozoon produces a change in the cytoplasmic structure of the egg, the original radial being replaced by a bilateral symmetry.

A very good instance of this is the formation of the grey crescent in the Frog's egg, already alluded to (p. 7).

Another is provided by the Ascidian *Cynthia*, in the immature egg of which there is a peripheral yellow substance surrounding a central grey yolk; the large germinal vesicle is near the animal pole (Fig. 9). Upon the entrance of the spermatozoon—near the vegetative pole—the germinal vesicle breaks down and maturation occurs. The contents of the germinal vesicle are discharged as a clear substance into the cytoplasm. Meanwhile the yellow layer has moved to the vegetative extremity of the egg, followed by most of the clear substance, only a small portion of which remains near the animal pole surrounding the female pronucleus. The animal hemisphere is thus occupied by the grey yolk. The egg has still a radial symmetry about its axis, but this is now replaced by a bilaterality, for the yellow and clear substances move to one side below the equator: this will be the posterior end, while the grey yolk, thus displaced, moves into the opposite side of the vegetative hemisphere. The pronuclei meet on the posterior side in the clear area, but finally move, taking this substance with them, into the animal hemisphere. We shall see that this bilateral symmetry of the egg is identical not merely with the symmetry of the bilateral cleavage but also with that of the embryo.

Other instances are known of the spermatozoon producing

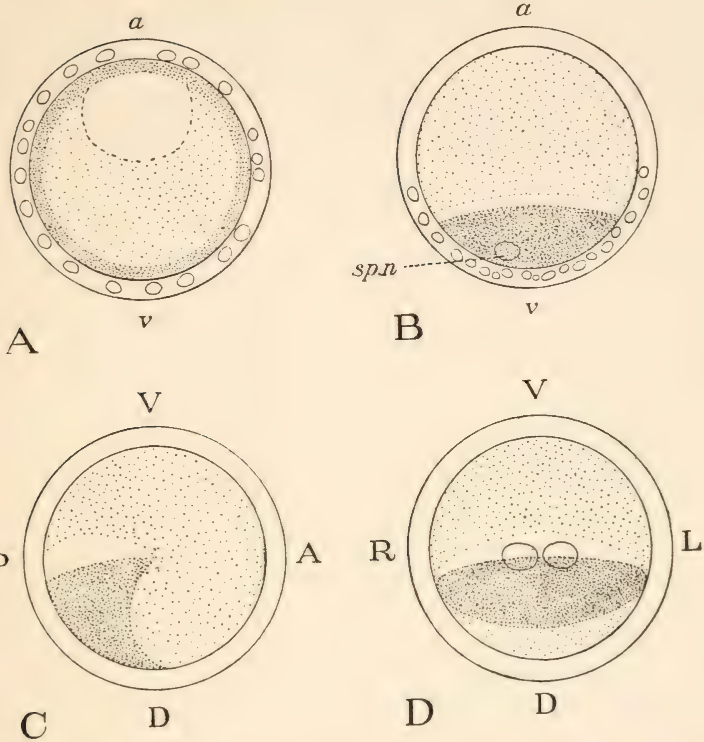


FIG. 9.—Normal development of the egg of *Cynthia partita*. Maturation and fertilization. (After Conklin.)

A, Unfertilized egg before the fading of the germinal vesicle (clear), showing central mass of grey yolk (lightly dotted), peripheral layer of yellow protoplasm (thickly dotted), test-cells and chorion.

B, After the entrance of the spermatozoon the yellow protoplasm has streamed to the vegetative pole (*v*); in it, excentrically, is the sperm nucleus (*sp.n*). The clear protoplasm derived from the germinal vesicle partly forms a layer over the yellow protoplasm, in part remains at the animal pole (*a*). The grey yolk occupies the animal part of the egg.

C, The yellow and clear protoplasmic substances have both streamed to what will be the posterior side (P). A, anterior, V, ventral (animal pole), D, dorsal (vegetative pole).

D, View of the same egg from behind. The two pronuclei are seen side by side in the clear area. R, right; L, left.

a similar alteration in egg structure; future investigations must show how far it is of general occurrence.

In a later lecture we shall see that the cytoplasmic substances so rearranged are causally related to the development of the various parts of the embryonic body, are in fact determinants of inheritance.

CHAPTER II

CLEAVAGE

The fertilized ovum is ready to develop, and the first step in development is segmentation or cleavage.

The segmentation of the ovum may be said to present to us, on the whole, four problems. The first of these is to discover the reason why the egg divides at all. The second is to find out why, when it does divide, it exhibits its own particular pattern of cleavage. In the third place we have to inquire into the causes which bring segmentation as such to an end. The fourth question asks whether cleavage is or is not in itself a process of differentiation.

These problems we shall discuss in order.

1. It is hardly necessary to say that any knowledge we may have of the nature of the causes which provoke the division of the egg is due to the genius of the American physiologist Jacques Loeb, for Loeb has shown that it is possible to replace the stimulus which is normally given to the egg by the spermatozoon, by the action of a solution, that is, by a physical or chemical agency. It is true that others had previously attempted in this way to cause the egg to divide, notably Hertwig and Morgan, but these met with little success. To Loeb belongs the honour of the achievement.

Since the methods employed have undergone some modification we may briefly recapitulate the history of these experiments.

The subject was in the first instance the egg of a Sea-urchin (*Arbacia*, and in later experiments, *Strongylocentrotus*), and it was found possible to incite it to segment and develop by temporarily immersing it in sea-water to which

some magnesium chloride had been added. The superiority of the results obtained by the use of this salt led Loeb at first to attribute the effect to some specific activity of the magnesium ion.

Since however the solution used was hypertonic to sea-water, the question arose whether the determining cause might not be the increased osmotic pressure. Experiments were therefore instituted in which the eggs were subjected temporarily to the action of solutions of a number of substances in sea-water, the solutions being all hypertonic to sea-water but all isotonic with one another. The substances employed were not only salts like magnesium chloride, sodium chloride, and potassium chloride, but also non-electrolytes such as cane-sugar and urea. After temporary immersion in any of these solutions the eggs segmented and developed. The hypothesis was then adopted that the increased osmotic pressure, resulting in a withdrawal of water from the eggs, was responsible for the result. The hypertonic solutions must be alkaline and contain oxygen.

It is to be noticed that development was not perfect in any of these cases, for the fertilization membrane was not formed, the cleavage was very irregular—simultaneous division into several cells, and multipolar mitoses occurred—and the larvae sank to the bottom instead of swimming at the surface.

Further investigation showed however that solutions which were physically isotonic did not produce precisely the same effect, were not in fact physiologically equivalent, and conversely that solutions which had the same physiological effect had not the same osmotic pressure, as may be seen from the accompanying table (Table IV).

TABLE IV.

Solution.	Optimal concentration in gram-molecules.	Dissociation.	Osmotic pressure in atmospheres.
Cane Sugar	0·96 m.	—	21·53
Grape Sugar	1·04 m.	—	23·33
$CaCl_2$	0·50 m.	64 %	25·57
$MgCl_2$	0·49 m.	70 %	26·47
LiCl	0·74 m.	66 %	27·59
NaCl	0·79 m.	71 %	30·28
KCl	0·78 m.	77 %	30·95

The reason for this is that the eggs are not surrounded by a semi-permeable membrane or coating, but that their surface is permeable to these different substances in varying degree. Since then the eggs admit the entrance of the substances to a greater or less extent, the effect may be due in part at least to some other cause than the osmotic pressure of the solution. And indeed the rôle attributed to these solutions in the later, improved method, in which they are also employed, is different.

In the improved method the egg is first submitted to the action of an agent which incites the formation of the fertilization membrane, a feature characteristic of the normal process, but absent, as we have seen, in the earlier experiments.

The brothers Hertwig had, many years before, caused the formation of this membrane by the use of chloroform. Loeb was thus led to try esters and fatty acids, and of the latter butyric acid was found to be the most reliable.

The procedure adopted is briefly as follows: the eggs are placed for a short time (from $1\frac{1}{2}$ to $2\frac{1}{2}$ minutes) in sea-water containing a little butyric acid (55 c.c. sea-water + 3 c c. $\frac{n}{10}$ butyric). They are then removed to sea-water (which must be alkaline), washed, and kept in sea-water (alkaline) for from 15 to 20 minutes. The membrane is now formed in precisely like manner to the normal membrane. Should the eggs remain longer in the sea-water, however, cytolysis occurs; that is, droplets of clear cytoplasm begin to protrude from the surface, are separated off, and this continues until the whole egg is broken up into fragments. To prevent this some corrective must be employed. The eggs are accordingly placed either in a solution hypertonic to sea-water (made usually by the addition of NaCl, for example, 50 c.c. sea-water + 8 c.c. $2\frac{1}{2}n$ NaCl), which must also both be alkaline and contain oxygen, or in a dilute solution of potassium cyanide (for instance, 50 c.c. sea-water + 2 c.c. $\frac{1}{20}$ per cent. KCN).

The eggs, after remaining some time (half an hour or more) in the corrective solution, are finally retransferred to sea-water,

where they segment normally[1] and develop into swimming larvae.

The larvae produced by this improved method of artificial parthenogenesis may be reared through the metamorphosis and reach the adult condition. It should not be forgotten that the credit of first accomplishing this belongs to Yves Delage (who used, however, a different method), and that at a time when the means now employed at Plymouth for rearing larvae had not come into use. The individual first successfully raised by Delage was a male, with ripe generative products.

The success of Loeb's experiments naturally gave an impetus to the inquiry into this problem, and artificial parthenogenesis has been induced in the eggs of many animals, by many methods in the hands of various investigators.

Thus, Delage used for Sea-urchins (*Strongylocentrotus*) acids and alkalis alternately, for *Asterias*, carbon dioxide: Lefevre has employed acids for *Thalassema*, Loeb, saponin for *Polynoe*. Osmotic pressure (hypertonic solutions) has given good results with *Mactra* (Kostanecki), *Chaetopterus* (Loeb, Mead), *Amphitrite* (Scott), *Nereis* (Fischer), *Ophelia* (Bullot). Greeley has found cold sufficient, Lillie heat for *Asterias*, while Mathews used merely mechanical agitation for the same form. Lastly, in Vertebrates, Bataillon found that while hypertonic solutions would cause the eggs of *Petromyzon* and *Rana* to pass through only a few of the cleavage divisions, the eggs of the latter might be stimulated not only to segment but to develop by being punctured with a fine needle. From the punctured egg a perivitelline fluid was exuded, in it a grey crescent appeared at the normal time, the eggs segmented and a few of the larvae produced lived almost to the metamorphosis.

Though the very diversity of the means—mechanical, physical, chemical—by which artificial parthenogenesis may

[1] That is, the first three furrows are meridional, meridional, and equatorial. We are not told whether in the next division the characteristic mesomeres, macromeres, and micromeres are produced.

be brought about suggests that the mechanism of the process is not to be understood by reference to the factors involved in one method alone, the theories which his own experiments have induced Loeb to adopt, are well worth a brief discussion.

As Loeb points out, the whole action falls into two phases. In the first the egg is subjected to the influence of a substance which not only determines the formation of the membrane but also sets in motion certain changes which ultimately lead to the destruction of the egg by cytolysis. In the second phase it is rescued from the disastrous effects of the first solution by being immersed in a second.

It appears that the chain of events set in motion by the first solution (the butyric or other fatty acid) consists in part at least of the oxidation of substances in the egg. The membrane is also formed, but the mechanism of that is another process, to which we shall return in a moment.

It is known (Warburg) that the unfertilized egg undergoes a very slow oxidation, which is increased many times not only by normal fertilization, but also by the use of the membrane-producing reagent. It is these oxidations that lead to cytolysis, since by them are formed certain decomposition products which are toxic to the egg.

The function of the second reagent is either to stop this harmful oxidation or else to counteract it. The first is accomplished by the use of potassium cyanide, which inhibits oxidation generally, and the part played by this substance in the rescue of the egg from death is not hard to understand. But it is more difficult to form a conception of the rôle of the alternative agent, namely a hypertonic solution, for, as pointed out already, this must be alkaline but must also contain oxygen. If potassium cyanide be added to the hypertonic sea-water, or if the oxygen be removed and replaced by hydrogen, then the solution becomes ineffective or at least requires a much longer sojourn of the eggs in it before they can be made to develop on being replaced in sea-water.

Hence the evil effects that follow on the oxidations incited by the first solution are counteracted by another oxidizing

agent, which is supposed to render the egg immune, or to carry off the toxic substances.

It must be admitted that theory here leaves us in the lurch.

It is interesting to notice that the same two phases, destructive, involving the formation of the membrane, and counteractive, permitting of segmentation and development, can be distinguished in fertilization by a spermatozoon. When the eggs of a Sea-urchin are inseminated with the sperm of a Starfish, they all form membranes. This is due to the contact of the sperm with the egg surface. But while all the sperms touch the egg, they are not all able to enter and complete the process. If so able, then the egg develops normally; but if the sperm remain outside, the egg undergoes cytolysis, from which, however, it may be saved by timely treatment with hypertonic sea-water. On removal to ordinary sea-water, it develops. The spermatozoon therefore normally conveys to the egg first the membrane-forming substance and then the counteractor.

While we still await a more satisfactory explanation of the workings of these stimulants to parthenogenesis, we have been able to gain rather more insight into the mechanism by which the membrane is thrown off.

The hypothesis adopted by Loeb is based on the fact that the agent employed (a fatty acid) is lipoid-soluble, and upon a certain conception of the structure of the egg-cytoplasm. This structure is supposed to be alveolar, and the contents of the alveoli are supposed to be prevented from coalescing with one another by a coating of a lipoid (lecithin perhaps). The fatty acid destroys this coating, the superficial alveoli coalesce (the fatty acid having only penetrated a short distance below the egg surface), absorb water, and the accumulating perivitelline fluid, being hypertonic to sea-water, throws off the surface-layer of the egg—inter-alveolar substance—as the membrane.

When the membrane is fully expanded the perivitelline fluid is practically pure sea-water. If there be added to the sea-water a substance which increases the osmotic pressure,

but to which the membrane is impermeable (Mammalian blood-serum, for instance), the membrane at once collapses. Should the lipoid-soluble reagent be allowed to penetrate still further into the egg, the deeper parts of the latter become liquefied and cytolysis occurs.

It is, however, doubtful whether this hypothesis is entirely satisfactory. Solution is a physical process, but the magnitude of the temperature coefficient or quotient points to a chemical reaction, as Harvey and indeed Loeb himself have shown.

The temperature quotient for an interval of 10° C. for a chemical reaction is at least 2: for a physical action it is much lower.

The following table shows that its magnitude for this reaction is about 2 (taken from Harvey, for various Sea-urchins).

TABLE V.

Exposure in minutes.	Temperature.	Optimum concentration in c.c. of $\frac{n}{10}$ acetic to 50 c.c. sea-water.
$1\frac{1}{2}$	23°	6
$1\frac{1}{2}$	33°	2–3
3	23°	3–4
3	33°	$1\frac{1}{2}$–2
6	23°	3
6	33°	$1\frac{1}{2}$

The solution of the lipoids by the fatty acid must then be rejected. A change in the surface-tension of the egg might be suggested, but surface-tension is not altered much by temperature, nor is the rate of diffusion, nor the dissociation of the fatty acid.

A chemical explanation is therefore put forward, namely, that the acid combines with the protein at the egg-surface, and that the compound so formed is more permeable than the cytoplasm of the unfertilized egg. It is supposed then that substances (protein) pass out through this surface-layer and are immediately coagulated by the water to form the membrane. Or else it may be imagined that the altered surface-layer itself becomes the membrane. Through this membrane

—presumed to be permeable to water and salts, but impermeable to the proteins and sugars in the egg—sea-water is then absorbed and the membrane raised from the surface.

There are facts which support this view. In the first place it is known that all haemolytic agents—all agents that cause the diffusion of haemoglobin from a blood corpuscle—are also capable of inciting the formation of the membrane in these ova. Such are electricity, heat, hydroxyl ions, hydrogen ions, distilled water, fat solvents, bile-salts, soaps, glucosides, such as saponin, digitalin, solanin, and blood-sera. Haemolysis must depend on an alteration of the permeability of the surface-layer.

In the second place Lillie has shown that the order of effectiveness of neutral salts of potassium and sodium in causing membrane formation is the same as the order of their effectiveness in causing the liberation of the red pigment from the egg of the Sea-urchin *Arbacia*, and the latter must depend on an increased permeability of the peripheral layer of the egg.

The following table gives the salts in the order of effectiveness:

$COOCH_3$ is least effective
Cl
Br
ClO_3
NO_3
I
CNS is most effective.

The hypothesis seems peculiarly applicable to *Nereis*, for here, as we have already seen, there is a preformed membrane which becomes separated from the egg by a perivitelline fluid as the result of insemination. This fluid is simply sea-water which passes through the membrane and fills the superficial alveoli from which their gelatinous contents have escaped and diffused out. It will be noted that the membrane must have become more permeable, and that the walls of the alveoli remain, whereas on the lipoid-solution theory they should disappear.

The membrane formed by these agents is apparently exactly like that produced normally by the spermatozoon. We have now to inquire what resemblance the division apparatus of these parthenogenetic eggs bears to that which is made by the sperm-centrosomes in a fertilized egg.

The details have been revealed to us by the investigations of Wilson into the cytology of magnesium chloride eggs, of Hindle into that of those stimulated by the later butyric acid method.

The two accounts are in essential agreement as to the origin of the division centres, but we shall follow Hindle in the main, since the segmentation of these eggs at any rate approaches the normal.

The species used was *Strongylocentrotus purpuratus.*

The following changes occur in the short interval (fifteen to twenty minutes) between the removal from the butyric and the immersion in the hypertonic solution.

The membrane having been thrown off, the nucleolus of the female nucleus, previously chromatic, loses its affinity for basic dyes, and its definite shape; it may fragment. Meanwhile there appears a clear perinuclear zone of cytoplasm from which radiations pass out in all directions. In the hypertonic solution the nucleus enlarges, while the perinuclear zone almost disappears, but, on removal to sea-water, reappears, while the nucleus enlarges still more.

Two cleavage asters are now developed with a spindle between them; each contains a centrosome. They are formed by division of one aster and centrosome; Wilson has shown that the centrosome originates from, or at least at the surface of, the nucleus. There are also in the cytoplasm independent cytasters, each with its centrosome, which in this case is not of nuclear origin.

If exposure to the hypertonic solution is not too long, these cytasters disappear and division takes place across the equator of the 'fertilization' spindle, the female nucleus having first broken up into chromosomes, which are present in the reduced number (n), a number which persists at least as far as the blastula stage.

If, however, exposure to the hypertonic solution is too prolonged, the cytasters become very numerous, and united by spindles to one another as well as to the cleavage amphiaster. The chromosomes become scattered not only on the cleavage spindle, but on the other spindles too, and division occurring in all the equators of the multipolar figure, the egg is irregularly divided into several cells at once, as was always the case in the earlier experiments with magnesium chloride. Since division is not restricted to those spindles on which chromosomes are cast, but may occur in their absence, it happens that some of the cells produced are enucleate.

The centrosomes seen in these ova—not only those in the cytasters, but also those in the cleavage asters—are new formations. The foci for the formation of the cytasters may possibly be the chromatic particles in the cytoplasm (remains of the 'yolk-nucleus'), while the development of centrosome from the nucleus is paralleled by the mode of origin of the definitive centrosome in ordinary fertilization. There seems to be no ground for believing that the centrosome of the maturation divisions persists and is revivified.

In other cases also which have been examined [the Echiuroid Worm *Thalassema* (Lefevre), the Polychaet *Amphitrite* (Scott)] normal cleavage seems to depend on the presence of one amphiaster or cleavage spindle. In *Thalassema* the two centres with their asters appear simultaneously upon the nuclear membrane.

It appears therefore that just as the development of a division apparatus in ordinary fertilization is a necessary condition of cleavage, so also in artificial parthenogenesis a bi-polar spindle is a pre-requisite of normal, that is, binary segmentation. The centrosomes about and between which this apparatus is produced in the cytoplasm are in the first case of male, in the second of female nuclear origin.

In conclusion, a word may be said of the part played by this spindle in the division of the nucleus and the cell. In mitosis the chromatic material of the nucleus (which alone is divided) is thrown into the form of chromosomes. These bodies

divide independently, they are not divided by the spindle. The function of the spindle-fibres is merely to pull apart their halves. But the spindle does take an active part in cell-division. In plants the cell-plate, the future cell-wall, arises by equatorial thickening of the spindle-fibres, and there is a parallel phenomenon in the animal cell, for after the separation of the daughter chromosomes there arises a plate of material in the spindle equator which has a less surface-tension than the remainder of the cytoplasm. It is the reduction of the surface-tension, or rather the greater surface-tension of the remainder, which pulls the two blastomeres apart. This not only appears to be a theoretical necessity but is experimentally demonstrable. For if a drop of rancid olive oil be floated on water, or better on a mixture of alcohol and water, and a thread soaked in weak potash be laid across a diameter of the drop, the soap now formed along this diameter having a less surface-tension than the remainder, the drop divides. The experiment is due to Robertson: it may be readily verified.

We have finally to inquire whether the last event of ordinary fertilization, the alteration of the egg symmetry, finds a parallel in artificial parthenogenesis. In only one sense, so far, has it at present been found possible to give an answer to this question, in the frog's egg, where the grey crescent appears as a result of fertilization on that side of the egg opposite to the point of entrance of the spermatozoon. Brachet has shown that in the eggs stimulated by puncture a grey crescent appears, and at the same time as in the controls, but that it bears no definite relation to the point of puncture, being variably on the same side, on the opposite side, or at right angles to the latter. There is on the other hand an invariable coincidence between the plane of symmetry of the egg, as thus defined, and the median plane of the embryo, for the dorsal lip of the blastopore always appears in the region of the grey crescent. Brachet is therefore driven to the somewhat remarkable conclusion that the unfertilized egg is only apparently radially symmetrical, and

really, though invisibly, bilateral. In ordinary fertilization this weak primary bilaterality is superseded by the far stronger bilaterality imposed by the sperm, a bilaterality which persists as that of the embryo. In artificial parthenogenesis the primary bilaterality remains, becoming manifest as the result of the stimulation.

It must be pointed out that the number of cases on which Brachet relies are really too few to support any safe conclusion, and further that the coincidence between the plane of symmetry of the fertilized egg and the median plane of the embryo is not absolute but only approximate, as the accompanying table of the frequencies of the angle between the two planes will readily show (Table VI). The eggs used in this experiment were placed on the slides with their axes vertical and the white pole below, to avoid any disturbing influence of gravity, and spaced to avoid the influence of pressure.

TABLE VI. FREQUENCY.

Angle.	Positive.	Negative.	Total frequency.
0°–15°	68	59	127
15°–30°	36	32	68
30°–45°	10	13	23
45°–60°	4	5	9
60°–75°	1	8	9
75°–90°	3	1	4
90°–105°	1	—	1
105°–120°	—	—	—
120°–135°	1	—	1
135°–150°	—	—	—
150°–165°	2	—	2
165°–180°	1	—	1

It is clear that while there is a very strong tendency for the two planes to coincide, it may happen that they diverge a good deal, even to the extent of 180°.

Some caution should therefore be exercised in drawing conclusions as to the coincidence of the planes of fertilization, egg-symmetry, and embryonic symmetry from a small number of observations.

2. The second problem presented by cell-division is concerned with the causes which determine the particular pattern of cleavage in each case.

First, however, a word on the conditions of there being a system of cleavage at all.

It is an established physical principle that drops of fluid will only cohere to form a system of drops without fusing with one another if they are coated with a layer or film which is insoluble both in the surrounding medium and in the fluid of the drops themselves. If the former condition be not observed then the drops separate, if the latter condition be absent then the drops fuse. In neither case is there a system.

A beautiful experiment of Herbst's has shown that the possibility of the blastomeres into which an egg divides forming a coherent system is governed by a like condition. Around and between the blastomeres there is visible in many cases (the Sea-urchin egg for instance) a definite coating film. This is insoluble in ordinary sea-water, but if the egg be placed in an artificial sea-water from which the calcium has been omitted, then the film is seen to become dissolved, and the blastomeres separate. So far, therefore, the blastomeres behave like drops of other fluids. We shall see now that the pattern assumed by the cells in cleavage may also be explained, in some cases at least, by reference to the principles of surface-tension.

There are three principal patterns of cleavage, the radial, the bilateral (including the iso-bilateral), and the spiral. The chief features of these types have been often described, but may be briefly recapitulated here.

The radial type of cleavage is distinguished by the fact that more than three, usually four or eight, surfaces of contact between adjacent blastomeres may meet in one line; for instance, in the four-celled stage of a Frog's egg the four surfaces in question intersect in the egg-axis, and so on. The planes of division in this type also either include, are parallel to, or at right angles to the egg-axis. The same must be said of cleavages of the second type, with the addition that they are symmetrically disposed on each side of one plane, often that of the first furrow, as in the Cephalopod egg (Fig. 10), but it may be another plane, for instance in *Ascaris* the plane including the first four blastomeres. In the iso-bilateral form there are, of

course, two such planes, for example in the Ctenophore egg, and the Teleostean egg. In the spiral type, firstly, the successive cleavages are oblique to the egg-axis; secondly, never more than three surfaces of contact intersect in one line, so that cross- or polar-furrows are developed between opposite blastomeres in for instance the four-celled stage; while in the third place, successive quartettes of micromeres are thrown off at

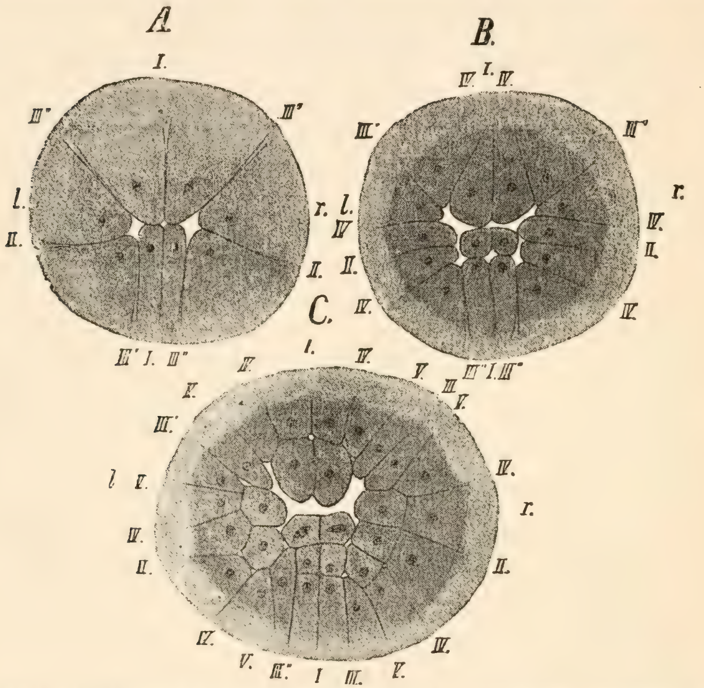


FIG. 10.—Three segmentation stages in the blastoderm of *Sepia officinalis*; the segmentation is of the bilateral type. *l*, left; *r*, right; *I–V*, first to fifth cleavages. The top sides of the figures are anterior. (After Vialleton, from Korschelt and Heider.)

the third and following phases of cleavage towards the animal pole, alternately in a right-handed and a left-handed direction (Fig. 11). In later stages spirally segmenting eggs assume the characters proper to the first and second types or may do so.

The law of alternation of direction of cleavage at successive divisions, just alluded to, holds good for a considerable time in

cleavage, and for the divisions of the micromeres of the various quartettes. It is, as a matter of fact, only a special case of a rule which is seen in segmentations of the other types also, namely, that successive divisions are at right angles to one another. It is known as Sachs' rule, since this botanist first formulated it for plant cell-divisions, and depends, in part at least, on the fact that the centrosomes, in preparation for the next division, divide at right angles to the previous spindle-axis.

The pattern of cleavage is conditioned obviously by the

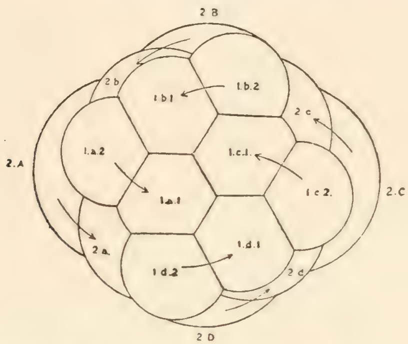


FIG. 11.—Diagram of a spirally segmenting egg in the 16-cell stage. 2 A–2 D macromeres; 2 *a*–2 *d* micromeres of second quartette; 1 *a* 1, 1 *a* 2–1 *d* 1, 1 *d* 2 micromeres of first quartette.

shapes, sizes, and arrangement of the cells, and these in turn on the rate and direction of division, and the movements of the cells on one another; and these again on the relation between the dividing nucleus with its centrosomes and the cytoplasm, and on the properties of that plasma and its inclusions.

These relations are expressed in certain rules, one of which, that of Sach's, has just been referred to. Another is Balfour's rule that yolk impedes division, hence the more rapid division and smaller size of animal cells in telolecithal eggs. Hertwig's rules state that the nucleus lies in the centre of the cytoplasm, and that the dividing nucleus or spindle elongates in the direction of greatest protoplasmic mass or, as Pflüger worded it, the direction of least resistance, resistance being offered by the yolk. These rules explain a good many of the features of division in the various types. The properties of the cytoplasm which play

a part in the ordering of the pattern appear to be, sometimes, the physical properties which they possess as liquids; for in spirally segmenting eggs, where never more than three surfaces of contact between adjacent blastomeres intersect in one line, making angles of 120° with one another, the cells are merely obeying the laws of surface-tension as enunciated by Plateau for systems of drops, for example soap-bubbles. In the other types of cleavage, it is true, these capillary laws are not obeyed, but even here as Roux has shown it is possible to cause drops of oil to imitate the arrangement of a radial system by enclosing them in a boundary; in the radial eggs the membrane may represent such a boundary.

More than this need not be said at the moment since these matters may be found discussed in the text-books.

3. Nuclear and cell-division continue of course throughout the life of the organism; and during all but the earliest stages of development they are accompanied by the processes of growth and differentiation. There is, however, an earliest stage of all in which the material of the ovum is simply cut up into small pieces, the cells, without the concurrence of any growth or of any differentiation other than the formation of the segmentation cavity, and such physical and chemical alterations as may be taking place in the blastomeres under perhaps the influence of their nuclei. This early stage is the stage of segmentation, and we have now to discuss the nature of those causes which bring segmentation as such to an end and determine the beginnings of differentiation.

Following out an idea which originated with Richard Hertwig, Boveri has suggested that initially the cytoplasm is too large for the nucleus, but that by the process of nuclear and cell-division the ratio of plasma to nucleus is reduced until it reaches a certain value, the attainment of which marks the end of cleavage, and the commencement of other processes.

The evidence adduced by Boveri in support of this hypothesis consists in the experimental demonstration that at a given stage of development the ratio in question has always a given

value, whatever be the initial size of the nucleus, which may be arbitrarily altered, while the cell-size is kept constant.

The number of chromosomes can be altered in the following ways:

1. In artificial parthenogenesis the number is n (maternal) (Thelykaryotic).

2. In merogony the number is n (paternal) (Arrhenokaryotic).

3. In monaster eggs the number is raised to $4n$ (Diplokaryotic). These are fertilized eggs in which the division of the centrosome has been delayed by shaking. The $2n$ chromosomes nevertheless divide, but return into the condition of a resting nucleus. From this, $4n$ chromosomes emerge when the centrosome does divide, and this number persists.

4. If the egg is kept for twenty-four hours in sea-water while the sperm is treated with dilute alkali, then upon fertilization the sperm-nucleus lags behind its centrosome, which divides to form a spindle in which only the female nucleus is included. The latter breaks up into chromosomes which are divided in the ordinary way, but the male nucleus passes undivided to one pole (Partial Thelykaryosis). Hence, after cell-division, one blastomere has $2n$ chromosomes (is Amphikaryotic) while the other has only n, which are maternal (is Thelykaryotic).

5. In dispermy it may happen that the spindles formed by the division of the two sperm-centrosomes remain apart, without uniting in the usual tetraster. The female nucleus lies in the equator of one spindle, together with one male nucleus, the other male nucleus lies in the other spindle. The two spindles are parallel, or may be, and division takes place in the plane including their equators, and also between them (if not at once, then eventually). Hence on one side of the egg there are Amphikaryotic nuclei with $2n$, on the other Arrhenokaryotic with n chromosomes. This is Partial Arrhenokaryosis.

6. The normal egg is Amphikaryotic, with $2n$ chromosomes.

The following examples will suffice to show how the relation between the number of chromosomes and the number of the nuclei is determined, cell (cytoplasm) size remaining constant.

A. Nucleate and enucleate egg-fragments are taken of the same size; both are fertilized, and develop. The number of cells in equal areas of the same germ-layer, at the same stage, is then counted.

For example:

	Nucleate fragment $2n$ (♀ + ♂).	Enucleate fragment n (♂).
Ectoderm of anal area .	167	317
Ectoderm of ciliated ring	86	163

B. The number of cells in larvae from monaster eggs is compared with that in normal larvae.

For example:

	Monaster ($4n$).	Normal ($2n$).
Blastula	23	43
Pluteus (animal ectoderm)	37	71

It is clear that at the same stage in larvae derived from the same original quantity of cytoplasm, the number of the cells in equal areas of the same tissue is inversely proportional to the number of chromosomes in the nuclei.

C. In partial Arrhenokaryosis and partial Thelykaryosis the larva is obviously composed of two regions (separated usually by the median plane) in which the size of the nuclei is different. The number of small (n chromosomes) nuclei is to the number of large ($2n$) as 2 : 1.

We turn to the relation between the number of chromosomes and the size of the nuclei as measured not by their diameters but by their surface-areas. The initial amount of cytoplasm in each case is constant as before.

A. Merogonic and nucleate egg-fragments.

	Nucleate fragment $2n$ (♀ + ♂).	Enucleate fragment n (♂).
In gastrula stage .	42	21
In pluteus stage .	46	25

B. Partial Arrhenokaryosis.

	$2n$ (♀ + ♂).	n (♂).
The two sides of the pluteus are compared	27	14

C. Partial Thelykaryosis.

	$2n$ (♀ + ♂).	n (♀).
The two sides of the pluteus are compared	30	14

D. Diplokaryosis; the monaster egg is compared with the normal.

Monaster ($4n$).	Normal ($2n$).
66	33

It is clear that the surface-area of the nucleus is directly proportional to the number of chromosomes contained in it.

It follows therefore at once that the size of the nucleus (as measured by its surface-area) varies inversely with the number of cells. But in equal areas of like tissues (which are of the same thickness) the number of cells must be inversely proportional to the size (volume) of the cells. Hence, the cell-volume is directly proportional to the surface-area of the nucleus, as well as to the number of chromosomes contained in it, or the ratio of plasma to nucleus has in the blastula stage, and again in each tissue at later stages, some constant value.

It is suggested that the attainment of this constant value as a result of the multiplication of the nuclei during segmentation brings this process as such to an end.

The converse of this experiment is seen if the number of cells is compared in larvae reared from fertilized nucleate egg-fragments of different sizes.

Two cases may be quoted.

	Ratios of surfaces of gastrulae.	Ratio of numbers of nuclei in gastrulae.
1.	1 : 6·5	1 : 1·48
2.	1 : 1·5 : 2·8	1 : 1·42 : 2·82

The number of nuclei being proportional to layers of equal thickness the cell-volume has, at a given stage, a constant value. This is, of course, a re-statement of Driesch's rule that in larvae developed from isolated blastomeres the number of cells and the surface-area are both directly proportional to the germinal value.

It will be observed that the value of the constant plasma-nucleus ratio has not been given by Boveri, and further that the actual volume of the cells has not been determined. This lacuna in our knowledge has been filled by the researches of Fräulein Erdmann, who has investigated in the same form as that employed by Boveri (*Strongylocentrotus lividus*) the changes in dimensions undergone by nucleus, cell, and chromosomes, during early development.

In the accompanying table (Table VII) the results for one temperature—10° C.—are given, for a series of stages.

TABLE VII.

Stage.	Volumes in cubic μ of Nucleus.	Cell.	Chromosomes.
2 cells	10037·0	106250·0	19·17
4 cells	1605·8	51063·0	10·83
8 cells	1081·0	26290·0	8·32
16 cells	837·8	9973·0	7·24
32 cells	803·6	6023·0	5·46
64–132 cells	529·7	2685·5	4·51
Blastula 1	460·5	1343·0	3·59
Blastula 2	332·4	549·7	2·77
Gastrula 1	117·4	292·5	1·92
Gastrula 2	62·9	180·7	1·00
Pluteus	28·7	118·0	0·41

It seems therefore that not only the cell-volume but also the nuclear volume decreases during segmentation, gastrulation, and the development of the larva, and it is obvious that the nucleus does not grow to the original size after each division. As we should expect from Boveri's work, diminution in the volume of the chromosomes is accompanied by decrease in the size of the nucleus; since, according to Boveri, the size of the nucleus depends on the number of the chromosomes, originally given to it, and the mean size of the paternal and maternal chromosomes is at least nearly the same.

At higher temperatures (15°–16° and 20°) the same diminution of the nucleus and its chromosomes is seen: the absolute values for any stage diminish as the temperature rises. At one and the same stage the number of cells is greater, the higher the temperature. The total amount of chromatin present in the embryo at any one stage (blastula or gastrula) is, however, constant at all temperatures.

From the data the ratios are at once obtained (Table VIII).

TABLE VIII.

Stage.	Ratios of volumes. $\frac{\text{Cell.}}{\text{Chromosome.}}$	$\frac{\text{Cell.}}{\text{Nucleus.}}$	$\frac{\text{Nucleus.}}{\text{Chromosome.}}$
2 cells	348·0	10·5	32·8
4 cells	294·0	31·8	9·3
8 cells	187·0	24·3	8·1
16 cells	86·0	11·9	7·2
32 cells	68·0	7·4	9·2
64–132 cells	37·0	5·0	7·3
Blastula 1	23·0	2·9	8·0
Blastula 2	12·0	1·6	7·5
Gastrula 1	9·4	2·5	3·9
Gastrula 2	11·2	2·9	3·9
Pluteus	19·0	4·2	4·3

The ratios given are for a temperature of 10°, but the same diminution with a slight rise at the end occurs at the higher temperatures. The final values reached are practically the same at all temperatures, and this suggests some causal relation between the amount of chromatin and the size of the nucleus and cell. The ratio of plasma to nucleus diminishes, as originally suggested by Boveri, but not at anything like the rate which a growth of the nucleus to its original size after each division would involve. At a low temperature this ratio reaches a lower value, that is the nucleus is relatively larger than at a high temperature. This is in accordance with a rule which the studies of R. Hertwig and his pupils on Protozoa seem to have established.

Lastly, the ratio of the surface-area of the nucleus to the chromosome volume does not remain constant, but diminishes and increases again.

Table IX.

Ratio of $\frac{\text{nuclear surface}}{\text{chromosome volume.}}$

Stage.	10°	15°	20°
2 cells	7·8	6·4	7·4
8 cells	3·6	3·6	3·8
32 cells	4·6	4·4	6·4
Blastula 1	5·0	2·6	2·0
Gastrula 1	3·6	2·2	2·0
Pluteus	6·8	6·0	5·2

As we have already seen, the nuclear surface diminishes as development proceeds, though of course not as fast as the nuclear volume. The nuclear surface, therefore, is determined neither by the number nor by the volume of the chromosomes taken alone.

This, however, does not necessarily invalidate Boveri's statement, which was based on a comparison of nuclear surfaces at corresponding stages with a varying number of chromosomes, not on a comparison of successive stages with the same number of chromosomes.

In the studies just considered the plasma whose volume is determined includes the yolk-granules as well as the actual living cytoplasm. It is the great merit of Conklin to have attempted to measure the volume of the cytoplasm as distinct from the yolk, at successive stages, and in the different cells.

The egg used was that of *Crepidula*, a Mollusc, which segments spirally. As segmentation progresses the plasma increases at the expense of the yolk.

The table gives the value of the nucleo-plasma ratio $\left(\frac{\text{nuclear volume}}{\text{plasma volume}}\right)$, as calculated from measurements taken when the nuclei first become spherical after division.

TABLE X.

Cell.	Ratio.
Before cleavage	1:27·5
AB or CD	1:13·5
A, B, C or D	1:14·5
$1\,A-1\,D$	1:12·7
$1\,a-1\,d$	1:14·5
$2\,A-2\,D$	1:12·7
$2\,a-2\,d$	1:25·6
$1\,a^1-1\,d^1$	1:35·7
$1\,a^2-1\,d^2$	1:14·6
$3\,A-3\,D$	1:1·1
$3\,a-3\,d$	1:14·5
$2\,a^1-2\,d^1$	1:10·3
$2\,a^2-2\,d^2$	1:10·3
$1\,a^{1\cdot1}-1\,d^{1\cdot1}$	1:14·6
$1\,a^{1\cdot2}-1\,d^{1\cdot2}$	1:7·0

There are great differences, it will be seen, in the value of the ratio in different parts of the egg. The nuclear size is apparently determined partly by the length of the resting period—the value of the ratio increases where this is prolonged, as in $3\,A-3\,D$—partly by the amount of plasma present, and partly by the number of chromosomes. The third factor is of course constant in all the instances quoted in the table, but Conklin has shown that the chromosomes may be scattered by the use of hypertonic solutions, and that the nuclei so formed from less are smaller than those formed from more chromosomes. The influence of the second factor can be demonstrated by experiment, for if the egg be centrifuged the yolk may be unequally distributed between the first two blastomeres. The blastomere with the larger share of cytoplasm has also a larger nucleus and a larger centrosome.

The mean value for the nucleo-plasma ratio of all the blastomeres is 1:15. Measurements made on adult tissue

cells—intestinal epithelium, gastric epithelium, liver epithelium, ectoderm, epithelium of gills, ganglion cells—give a mean ratio of 1 : 10·5. There is therefore apparently an increase.

The mean volume of all the nuclei does not, however, increase to its original size after each division, but diminishes at first to reach its original value once more by the 70-cell stage. The total amount of nuclear material has by this time therefore increased. In the Ascidian *Cynthia* there is similarly an eventual increase in the total volume of nuclei in spite of the diminution of the mean volume. These results are in exact agreement with those obtained by Fräulein Erdmann for the Sea-urchin.

4. The fourth question is whether the nuclear and cell-division of cleavage are themselves processes of differentiation.

According to a well-known theory, which is or rather was associated with the names of Roux and Weismann, while the cytoplasm of the ovum was regarded as isotropic or equivalent in all its parts, the internal causes of differentiation were placed in the nucleus, that is, the determinants or different materials on which the appearance in the offspring of the inheritable characters ultimately depend, were imagined to reside in the chromosomes of the nucleus, but to be gradually separated from one another in successive divisions. Nuclear division in other words was qualitative, and through its agency the qualitatively different determinants were distributed to the different cells, there to call forth in the cytoplasm the histological characters to which each was appropriated, the characters which are to the observer the actual sign of differentiation, which is therefore actually produced by cell and nuclear division, and would not occur without it. Further, each cell having received in this way certain determinants and those only, can alone give rise to certain structures, and the causes for the production by it of those structures lie wholly within itself, that is, in its nucleus. Development is therefore a process of self-differentiation of each part of a mosaic-work.

This theory was founded upon an observation and an experiment made by Roux upon the egg of the Frog. Roux believed that observation told him that the first furrow of the egg invariably coincided with the median longitudinal plane of the embryo, the second with the transverse plane (putting those cases aside in which the first furrow by an 'anachronism' occupied the position of the second). Hence of the two blastomeres one contained the determinants for the right half, the

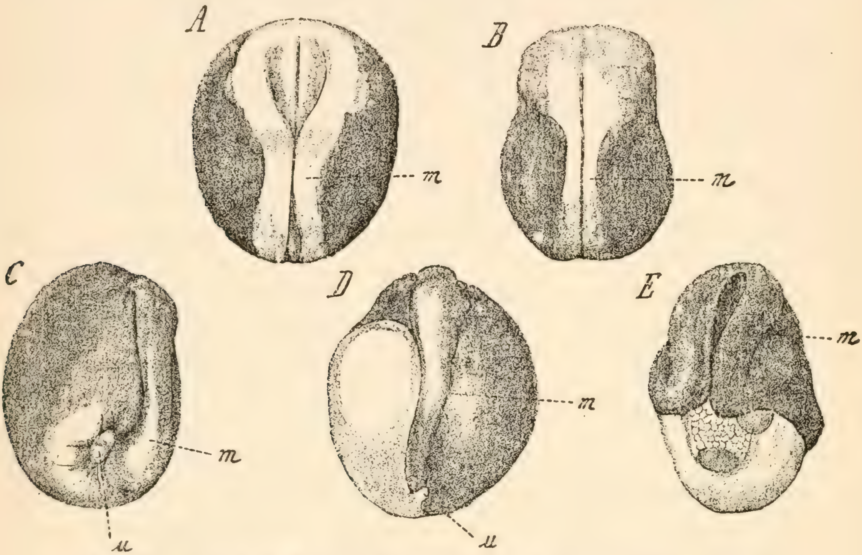


FIG. 12.—*A* and *B*. Normal Frog embryos with medullary folds (*m*), open (*A*) and closed (*B*). *C*. Hemiembryo dexter with almost complete post-generation of the ectoderm ; *u*, yolk-plug. *D*. The same, older, but with less post-generation. *E*. Hemiembryo anterior (?) with beginning post-generation. (From Korschelt and Heider, after Roux.)

other those for the left half of the body. This was confirmed by experiment, for when one of the blastomeres was killed by means of a hot needle the survivor was found to give rise to a half-embryo, right or left as the case might be (Fig. 12). Later investigation has, however, been able wholly to confirm neither the observation nor the experiment, while the institution of experiments on the ova not only of the Frog but of other animals has shown that a cell-division is not the cause of

differentiation in the cytoplasm, for the simple reason that that differentiation exists before cleavage takes place.

We may begin this discussion by a re-examination of the facts on which Roux's own theory was based.

The first statement is that the first furrow in the Frog's egg always coincides with the sagittal plane. This is certainly not the case, for if a sufficiently large number of eggs be examined it will be found that while in the majority the angle between the two planes is small, it is nevertheless possible for that angle to have any value.

One example will be enough. The following table gives the frequencies for different values of this angle under the most favourable circumstances, that is when the disturbing influences of mutual pressure and gravity have been removed.

TABLE XI.

Angle between First Furrow and Sagittal Plane.	Frequency.
−90°−75°	6
75°−60°	10
60°−45°	20
45°−30°	31
30°−15°	70
15°− 0°	82
+ 0°−15°	102
15°−30°	50
30°−45°	31
45°−60°	9
60°−75°	7
75°−90°	10
Total	428

The tendency of the two planes to coincide is measured by the standard deviation (σ), which in this case is $31{\cdot}45° \pm 0{\cdot}73$.

If now under the same conditions the standard deviation of the angle between the plane of symmetry (plane of the grey crescent) and the sagittal plane be determined it is found to have a value of $26{\cdot}80° \pm 0{\cdot}82$. In other words there is a greater tendency of the sagittal plane to coincide with the plane of symmetry than with that of the first furrow. The standard deviation of the angle between the plane of symmetry and the first furrow is highest of all, namely $34{\cdot}46° \pm 1{\cdot}07$.

The relation between these planes may also be stated by

finding the value of the correlation coefficient (ρ). This is, between the plane of symmetry and the sagittal plane $0{\cdot}451 \pm 0{\cdot}035$, between the first furrow and the sagittal plane $0{\cdot}364 \pm 0{\cdot}033$, and between the plane of symmetry and the first furrow $0{\cdot}186 \pm 0{\cdot}043$. This confirms the first result. We shall return later on to the relation between the plane of symmetry and the sagittal plane, but for the present it is sufficient to say that a statistical inquiry does not bear out Roux's assertion.

In the second place Roux claimed to have produced from one of the first two blastomeres a half-embryo. It must be conceded that this sometimes occurs, but not always. Oscar Hertwig, repeating the experiment, found that frequently the living blastomere developed into much more than a half-embryo, being apparently only impeded in its differentiation by the presence of the inert mass of the other. Morgan suggested that the capacity of the survivor to develop into a whole depended on the position it occupied with regard to the dead cell; when the egg turned over so that the dead blastomere lay underneath the living, the latter became a whole, and it was indeed found by the same observer that if the egg were turned upside down, the living blastomere gave rise to a whole embryo. Precisely the same result is seen in the experiment, due to Schulze, in which each of the two blastomeres is made to develop totally and the egg to give rise to a double monster, by merely turning the egg upside down in the two-celled stage. The further analysis of this experiment, by Wetzel, shows that under the influence of gravity the contents of the blastomeres are redistributed, the heavy yolk-granules sinking, the lighter cytoplasm rising, until each blastomere has acquired a new polarity of its own; and in such a way that now the two polarities are opposed, the two blastomeres like two eggs united by their vegetative poles, and the two components of the double monster united by the persistent yolk-plugs of their dorsal sides. There can be little doubt that if it were possible to separate the two blastomeres completely, each would give rise to an independent larva, as happens in the Newt, where the separation can be

effected by means of a noose of hair tied round the egg in the first furrow (Fig. 13).

It is highly probable, however, that even then due attention would have to be paid to the position of the first furrow with regard to the grey crescent. For Brachet has shown that if one blastomere be killed, the fate of the other depends upon the angle made by the first furrow with the symmetry plane. When the planes coincide, the survivor becomes a right or left half-embryo; when the angle is 90° the survivor (when it

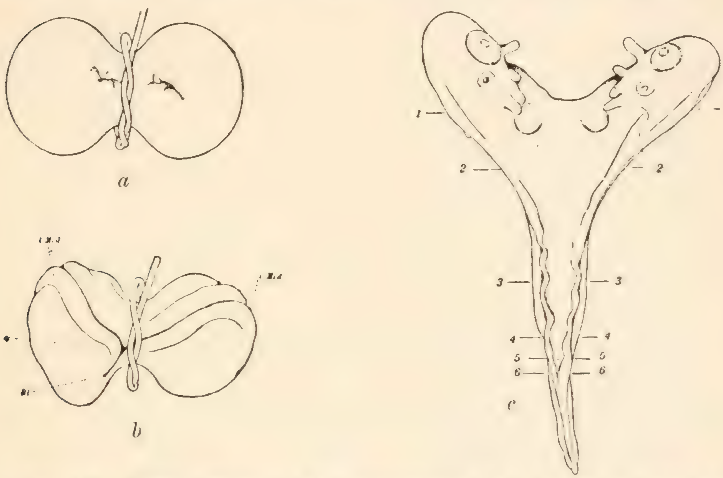


FIG. 13.—Three stages in the production of a double monster by strong median constriction of the Newt's egg. (After Spemann, 1903.) *a*. Beginning of gastrulation; there is a separate lip in each half. *b*. *l*. and *r*. *Med*., Medullary folds of left and right embryos; *, point where the medullary grooves separate; *Bl*, blastopore. *c*. The double-headed larva.

includes the grey crescent) becomes a postero-dorsal half-embryo; while when the angle is between 0° and 90° the survivor develops into an embryo which is defective on the right or left, anteriorly or posteriorly as the case may be.

This experiment demonstrates in the most convincing way the closer dependence of the embryonic symmetry upon the plane of egg-symmetry than on the first furrow.

In the Frog's egg, therefore, the factors that determine the symmetry of the embryo must be distinct from those that

determine the plane of the first and therefore of subsequent furrows, or as we may put it, the symmetry of segmentation, and in fact we know, with some degree of precision, the nature of these factors and the causes of their divergence.

The unfertilized egg has a radial symmetry about its axis: as a result of fertilization it becomes bilateral, since the grey crescent appears on the side of the egg opposite the point of entrance of the spermatozoon. It appears that the position of the grey crescent is determined not by the actual point of entrance of the spermatozoon, but by the position of the whole of the first part of the sperm-path, the entrance funnel. We also know that there is a considerable tendency for the median plane of the embryo to coincide with the plane of

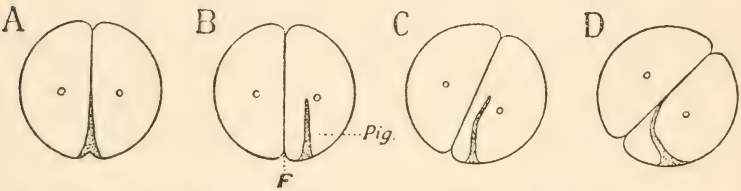


FIG. 14.—Roux's diagrams to show the relation of the sperm-path (*Pig.*) to the first furrow in the Frog's egg. In A the furrow includes the sperm-path, in B it is parallel to it, in C it is parallel to the inner portion of the path (copulation path), in D it includes only the very last portion of the copulation path. (From Korschelt and Heider, after Roux.)

embryonic symmetry, for the dorsal lip of the blastopore to be formed on the side of the grey crescent. We may assume then that the entrance of the spermatozoon imposes a bilateral structure upon the cytoplasm, a structure which persists as the bilaterality of the embryo.

In the second part of its path the sperm-nucleus moves to meet the female-nucleus. This second part may but need not lie in the same meridional plane as the first part (Fig. 14). When the first part is directed towards the egg-axis, and when the female pronucleus lies in the egg-axis, then both parts do lie in the same meridional plane, but when, as may happen, the female pronucleus is not in the egg-axis, or the sperm entrance path is not directed towards the axis, or possibly on account of other disturbances (gravity, &c.), then the meridional planes

of the two parts of the whole path diverge to a greater or less degree.

Now it is upon the second part of the path that the position of the first furrow depends, since it falls in the equator of the fertilization spindle, and the spindle is developed between the two centrosomes which are produced by the division of the sperm centrosome at right angles to that meridional plane which includes the line of union of the pronuclei. Hence, while the first furrow may and does indeed tend to coincide with the plane of symmetry and the sagittal plane, it need not do so.

One more point must be briefly alluded to. As has been mentioned the correlation between the sagittal plane and the plane of symmetry is not complete, even when disturbing agencies are removed. At the same time there is some correlation between the first furrow and the sagittal plane. It is worth while suggesting that the elongation of the spindle in a certain direction with the concomitant radiation of its asters, may itself impose some bilateral structure upon the egg material, and that the plane of embryonic symmetry may be a resultant of the separate influences exerted by the mitotic figure and the sperm-entrance.

With regard to the production of the grey crescent, which is due to the retreat of superficial pigment into the interior, it seems probable that this in turn depends on streaming movements in the cytoplasm set up by the aggregation of a watery material to form the entrance funnel.

We turn next to the theory of the qualitative division of the nucleus. Experiment compels us to reject this also. For if the egg of the Frog be subjected to pressure the sequence of the divisions is altered. When, for instance, the pressure is in the direction of the axis, while the first two furrows preserve their normal meridional directions at right angles to one another, the third furrows, instead of being latitudinal are again meridional or parallel to the first, while the fourth are latitudinal or parallel to the second. It follows that the arrangement of the nuclei produced by these successive divisions is also abnormal, and if the nuclei were really

qualitatively different, then the distribution of the determinants contained in them would be abnormal, and should cause an abnormal differentiation of the cytoplasm. As a matter of fact the egg develops into a normal tadpole.

The theory of the qualitative division of the nucleus is therefore proved untenable from more than one side. It is only fair to Roux to state that he has some time since abandoned an impossible position.

It only remains for us to review briefly the evidence of a like nature drawn from experiments on the eggs of other forms.

Experiments similar to that just quoted have shown that the pattern of cleavage may be altered by pressure without interfering with the normality of development in, for instance, the eggs of the Sea-urchin (Driesch) and of the worm *Nereis* (Wilson). In *Nereis* the egg segments into a flat plate of eight cells. On releasing the pressure, an octette of micromeres is formed instead of the usual quartette, and a normal trochophore is developed. The pattern of cleavage may be altered by other means. Thus in *Cerebratulus* (a Nemertine) in calcium-free sea-water the third division is meridional, then a first, and later a second, octette of micromeres are formed. Again, in certain cases the egg divides simultaneously into three, a meridional division produces six cells in a ring, sextettes of micromeres are then given off. In both cases a normal Pilidium is produced (Yatsu). In Sea-urchin eggs the blastomeres may be deranged by heat, shaking, and diluting the sea-water to any extent or almost so, without prejudice to the eventual normality of development.

In artificial parthenogenesis again, an irregular segmentation may be followed by a regular development.

Secondly, isolated blastomeres frequently segment as parts, that is, as though the remaining blastomeres were present. Thus a half-blastomere of *Echinus* gives rise to four mesomeres (animal cells), two macromeres, and two micromeres, and a quarter-blastomere to two mesomeres, one macromere, and one micromere. The isolated blastomeres of spirally

segmenting eggs (Nemertines, Molluscs) behave in the same way. The quarter-blastomere of *Patella*, for instance, produces one micromere of the first quartette, one of the second, and so on. But the fate of these isolated and partially segmenting cells differs, and depends indeed on the kind of material they contain. The half- or quarter-blastomere of a Sea-urchin egg, being produced by meridional divisions, possesses a proper share of all the materials present in the

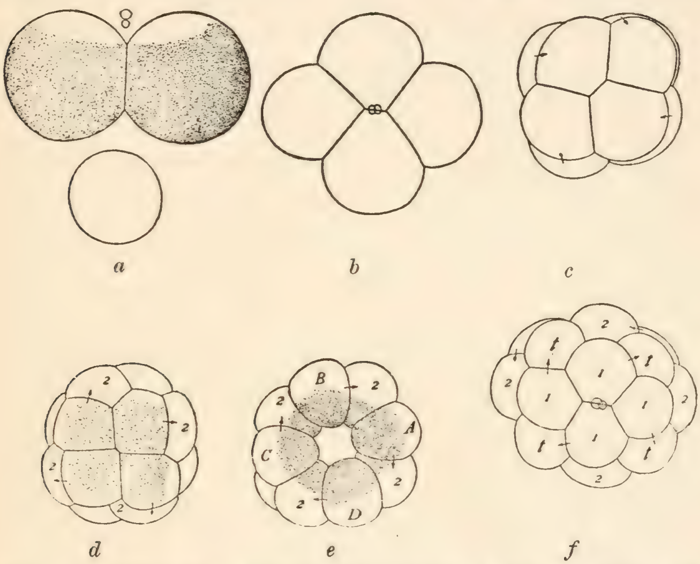


Fig. 15.—*Dentalium.* Cleavage after removal of the first polar lobe; *a*, first division: the isolated polar lobe is seen below; *b*, 4-cell stage, from animal pole; *c*, 8-cell stage, from animal pole; *d*, formation of second quartette, from vegetative pole; *e*, same stage as last, open type; *f*, same stage, from animal pole. (After Wilson.)

egg, since these are distributed symmetrically about the axis, and is able to develop into a whole pluteus. The half- or quarter-blastomere of the Nemertine egg can do the same. In the Mollusca (*Ilyanassa*, *Patella*) the developmental capacities of the cells are usually strictly limited (Crampton, Wilson), but in *Dentalium* while the *AB* half-blastomeres and the *A*, *B*, and *C* quarter-blastomeres not only segment but develop partially, the *CD* cell or the *D* cell, in which is

included the polar lobe, which other experiments have shown to contain the material necessary for the formation of the sense-organ and post-trochal region, develops into a complete larva.

In these cases a partial segmentation is followed by a total development.

The converse is seen in the total cleavage, at low temperatures, of blastomeres of *Ilyanassa* which are still unable to form whole larvae, and again in the complete segmentation of the Ctenophor egg from which a piece of the vegetative hemisphere has been removed, though the larva that is derived from such a fragment is defective (Driesch and Morgan).

Thirdly, it is well known that in the spirally segmenting eggs of Nemertines, Turbellarians, Molluscs, and Annelids, it is possible to trace the several organs and tissues of the embryonic body each back to an origin in some particular cell or group of cells in the cleavage system, and that as a general rule homologous organs, say the prototroch or the mesoderm, arise from cells which occupy identical positions and come into being by the same sequence of divisions in all cases. Thus the mesoderm usually is derived from the cell 4 *d*; 2 *d*, the first somatoblast, gives rise to the ectoderm of the ventral plate; the first quartette of micromeres is ectodermal and so forth. But there is no necessity that identical cells or cell-groups should have the same destiny, and there are many exceptions known to the general rule. One instance will suffice.

In the earthworm the nephridia, being derived from the ectoderm of the ventral plate can be traced back to 2 *d*, while in another Annelid, the leech, the same organs are mesodermal in origin and spring from 4 *d*. These need cause no perplexity, for, as we shall see more fully in a moment, the organs depend for their development upon the presence of some factor in the cytoplasm, but this factor (a material factor) need not be present in that position in which the organ to which it is appropriate will arise. Thus the factor which conditions the differentiation of nephridia, being in

both cases in the *D* quadrant, may readily in one case pass into the cell 2 *d*, in another into 4 *d*, and is then dubbed ectodermal in the one case, mesodermal in the other. It is none the less the same organ, homologous in the two forms, since its homology depends eventually on the conditioning factor, the cytoplasmic substance which is specific for it, and not, as has been sometimes supposed, on the cell into which that substance happens to pass.

And fourthly, it is possible to suppress cleavage without preventing differentiation. At least Lillie has been able, by the use of potassium chloride, to show that the unsegmented egg of *Chaetopterus* will put out cilia, while certain cytoplasmic substances undergo a change of position. In particular certain granules assume the position normally taken by them in the cells of the prototroch. It is interesting to note that in these ova the nucleus enlarges, so that the plasma-nucleus ratio is reduced.

But though the factors on which the pattern and symmetry of segmentation depend are distinct from those which condition the symmetry of the embryo, it must not be forgotten that they may coincide. Thus we have seen that in the Frog's egg, the plane of symmetry, the first furrow, and the sagittal plane may sometimes all be coincident, and there are other cases. In Amphioxus (Cerfontaine) and in Ascidians (Conklin) this occurs and again in the Cephalopod Mollusca, though it remains for a statistical examination to show that the coincidence is invariable. In spiral eggs also, the median plane of the embryo usually has a definite relation to the cleavage system, the *D* quadrant being as a rule posterior.

Experimental analysis, however, shows the difference. The causes which determine a particular pattern of cleavage, as we know, consist in the physical properties of the cytoplasm, and in the relation between the cytoplasm with its constituents and the dividing nuclei with their centrosomes. The causes of differentiation on the other hand, as we are now to see more fully, reside in the first instance in specific organ-forming cytoplasmic materials.

CHAPTER III

DIFFERENTIATION

We shall be obliged to limit ourselves to a discussion of the internal factors. These are to be sought, firstly, in the initial structure of the germ, that is, of the fertilized ovum, and, secondly, in the interactions of the developing parts.

I. The ovum comprises the cytoplasm and the nucleus, and each of these constituents has its part to play in the determination of inheritable characters.

A. The Cytoplasm.

The evidence which we have just been reviewing has shown us not only that the division of the nucleus is not qualitative, but also that the cytoplasm is not the homogeneous isotropic body imagined by the 'Mosaik-theorie', at least as originally propounded. We know now that the different parts of the cytoplasm are related causally to the formation of the various organs of the embryo, are therefore factors in differentiation and determinants of inheritance.

The testimony of experiment is conclusive on this point, since it is known that the abstraction of a certain part of the cytoplasm involves the absence of certain embryonic organs. As the facts are fairly well known they need only be briefly recapitulated here.

The development of experimentally isolated parts of the ovum—they may be pieces of the unsegmented ovum, or blastomeres removed during cleavage—has been observed in a number of forms, and it has been shown that while it is possible in certain cases to obtain a whole embryo or larva from such a portion, the capacity of a part to develop into a whole is conditioned by the structure of the egg-cytoplasm. Thus in the Hydromedusae among the Coelenterates this cyto-

plasm consists usually of a finely granular ectoplasm, and a coarsely granular endoplasm. The first four divisions—meridional, meridional, equatorial, and meridional—are all perpendicular to the surface, and each blastomere has a share of each of these two substances in the same proportions as they exist in the whole egg. Experiment shows that when separated from one another half-, quarter-, one-eighth-, and even one-sixteenth-blastomeres will develop into whole hydroids or medusae as the case may be. In *Carmarina*, however, there is in addition a jelly-plasm, a spherical hyaline mass placed excentrically near the vegetative pole. This is really a precociously secreted mesogloea and passes normally into the exumbrella of the medusa. Isolated half- and quarter-blastomeres, being produced by meridional divisions, naturally each include a portion of this as well as of the other two materials, and they can develop into whole medusae. But the one-eighth-blastomeres, which must either contain none or relatively too much of the jelly-plasm, can only develop partially. In *Aegineta*, another medusa, there is also a jelly-plasm in the egg, but centrally placed. The one-eighth-blastomeres are here totipotent.

Amongst Vertebrates it is possible to obtain a whole larva from each of the first two blastomeres in the Newt, at any rate when the first furrow lies in the (invisible) plane of egg-symmetry, and in the Frog we know that a double monster can be produced by turning the egg upside down in the two-celled stage, and so causing each blastomere, by rearranging its contents, to acquire a new polarity of its own. In both these cases, since the first furrow is meridional, each of the blastomeres has a share of the various substances of the cytoplasm which are placed symmetrically around the axis of the telolecithal egg. We should expect perhaps that after the third, latitudinal, division the potentialities of animal and vegetative blastomeres would differ. The evidence—slender though it is—shows that this is not altogether so, for a blastopore and archenteron may be developed not only from the four vegetative cells when the others have been destroyed (Morgan), but also from the animal cells alone (Samassa).

It is, of course, from the former that the structure in question is derived in the development of the whole egg.

We shall discuss the significance of this later on. It has been pointed out above that the egg-cytoplasm acquires its definitive bilateral structure as a result of fertilization. It is interesting that the removal of a part of the cytoplasm before the appearance of the grey crescent has far less serious consequences than after the bilateral symmetry has been established. In the first case the embryo is normal, in the second while it may be normal, it is frequently defective in certain respects or altogether unable to develop (Brachet).

In Amphioxus, Echinoids, and Nemertines the egg has a telolecithal structure and a polar radial symmetry. In Amphioxus the radial is replaced by a bilateral symmetry as a result of fertilization (Cerfontaine) exactly as in the Frog and in Ascidians (Conklin). At what moment the bilaterality is determined in the other two cases is not known.

In all three groups the first two and again the first four blastomeres have similar shares of the parts of the polar structure, since the first two divisions are meridional, and in all three isolated half- and quarter-blastomeres give rise to whole embryos or larvae of reduced size. But by compressing the egg of *Cerebratulus* at right angles to its axis the second division may be made equatorial; the quarter-blastomeres are then either animal or vegetative, and behave when isolated like the one-eighth-blastomeres of the normal egg (Yatsu). The next division, however, separates animal from vegetative one-eighth-blastomeres, and these are no longer totipotent, at least not invariably so. In Amphioxus neither the animal nor the vegetative cells can gastrulate; in the Nemertines the vegetative cells gastrulate but form no sense-organ, the animal cells form a sense-organ but no gut; and in the Sea-urchin the animal cells usually give rise to long ciliated blastulae, though they may gastrulate, while the vegetative cells develop more frequently into gastrulae with a pair of skeletal spicules.

There is therefore a difference between the cells derived

from the animal and from the vegetative hemisphere in respect of their developmental capacities.

In the Nemertines there is a similar difference between the capacities of animal and vegetative egg-fragments, removed prior to fertilization (Yatsu); the vegetative quarter of the egg gives rise to a larva without sense-organ, the animal fragment of about the same size, to a larva with defective gut and also without the apical organ; so that apparently the factor on which the development of this organ depends is somewhere in the centre of the egg, that is, not in its definitive position. Into that position it moves after the first cleavage, since removal of the animal regions of both blastomeres does not interfere with its development. At an earlier stage still (prior to the breaking down of the germinal vesicle) any nucleated piece of an ovum can give rise to a normal larva, whatever be the direction of the cut by which it is removed; that is, since the nucleus is in the animal hemisphere, a meridional, an oblique or an animal fragment.

The development of egg-fragments of Sea-urchins is said to be always complete, provided the fragment be not too small (Driesch); but this is a matter which requires re-investigation. In the Ctenophora the development of isolated blastomeres is always partial, at least in respect to the costae, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{3}{8}$, $\frac{10}{16}$ blastomeres giving rise to larvae with respectively 2, 1, 3, 5 costae, and so on. The stomodaeum, however, of $\frac{1}{2}$ and $\frac{1}{4}$ larvae is complete; and a $\frac{3}{4}$ larva has four and not merely three endodermal canals. In the Mollusca as a rule a cell when isolated gives rise to no more than it would have done had it remained in connexion with its fellows, except that the cell-mass produced from it may form a closed vesicle (for instance, isolated 1 *a*—1 *d* cells of *Patella* (E. B. Wilson)), but in those peculiar cases (*Ilyanassa*, *Dentalium*), where there is a polar lobe the cell in the two-celled stage or four-celled stage which possesses it, i.e. either *CD* or *D*, can produce a whole larva. Removal of the polar lobe from the whole egg involves absence of mesoderm in *Ilyanassa*, absence of the post-trochal region, and of the apical sense-organ in *Dentalium*. Removal of the lobe from the *CD* cells involves in the latter genus the same consequences, but

the larva reared from the lobeless *D* cell has an apical organ though still devoid of a trunk. The polar lobe of the egg, therefore, contains some factor on which the development of the apical organ, as well as that of the trunk, depends, and the factor for the sense-organ moves from its original position in the vegetative hemisphere to its definitive position at the animal pole between the first and the second divisions.

In *Ascaris* again where the cells from which certain organs come can be distinguished from the beginning the isolated blastomeres have only a partial development (Stevens). Thus S_1 (Fig. 5, p. 13) gives rise to an ectoblastic vesicle, P_1 to P_2 and *E M St* and the derivatives of these, and so on. Removal of one ectodermal cell (*A*) in the four-celled stage does not, however, very seriously interfere with the development of the remaining three into a normal embryo. Strictly speaking, the blastomeres are not isolated, but one or more is killed by ultra-violet light.

If, however, the egg be centrifuged it divides meridionally, and each cell then segments like a whole egg, with diminution of the chromosomes in the somatic cells. Lastly, in Ascidians the cytoplasm has a very definite structure (easily visible in *Cynthia*, only with the help of reagents in *Phallusia*), the parts of which assume their definitive bilateral arrangement as a result of fertilization. This bilaterality persists as that of the embryo, and since the first furrow (meridional) coincides with the plane of symmetry and the sagittal plane, and the subsequent furrows (meridional, latitudinal, and so on) have very definite positions with regard to the first, the various regions of the cytoplasm necessarily pass into particular cells. It is not therefore surprising that a cell should never be able, when the others have been killed, to give rise to any more than it would have done in the whole egg. As in *Ascaris* and in the Mollusca the potentialities of isolated cells are restricted *ab initio*.

While the cytology of the ovum shows us that its cytoplasm has always a certain structure, though different in different cases, a structure which assumes its definitive form during

maturation and, in some instances, fertilization, the experiments just reviewed demonstrate the necessity of the parts of this structure for the development of organs of the embryonic body; removal of this or that part entails the absence of this or that organ. The materials therefore on which the formation of these organs depends may be said to be preformed in

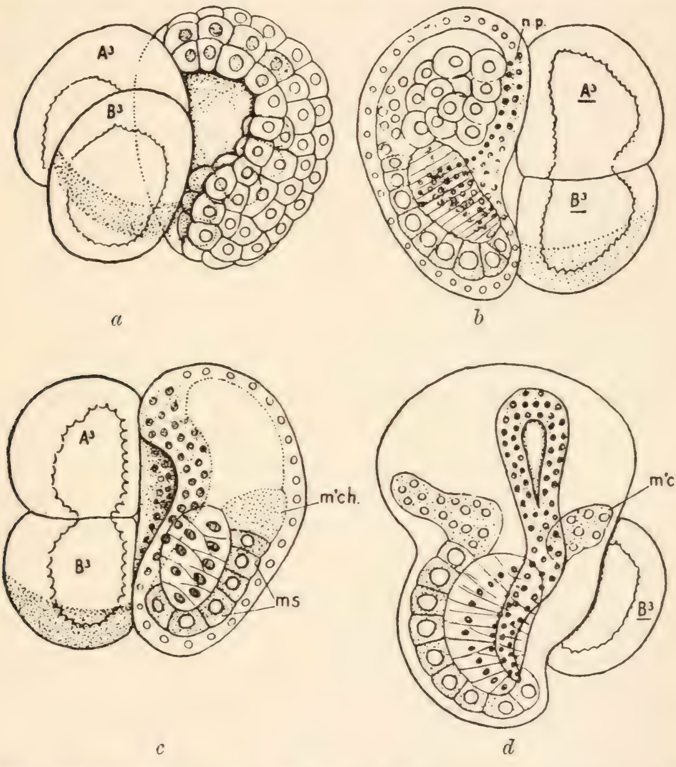


FIG. 16.—*Cynthia partita.* Half and three-quarter embryos. (After Conklin.) *a*, right half gastrula, dorsal view. The neural plate, chorda, and mesoderm cells are present only on the right side and in their normal position and numbers. *b*, left half of young tadpole, dorsal view. The notochord is normal except for size and number of cells; the muscle and mesenchyme cells are present only on one side; the neural plate (*n.p.*) is abnormal in form but not in position. *c*, right half of young tadpole, dorsal view; slightly younger than *b*. *m'ch.*, mesenchyme, *ms.*, muscle-cells. *d*, left anterior three-quarter embryo, dorsal view. The anterior half is entirely normal with anterior mesenchyme (*m'ch.*) on both sides. Posterior mesenchyme and muscle-cells upon the left side only.

the cytoplasm, though the organs themselves of course are not. But though preformed they are not prelocalized since—the apical sense-organ in *Cerebratulus* and *Dentalium*—they need not be initially present in their ultimate position.

On the other hand it is possible to divide the ovum into totipotent parts, which may be either fragments of unsegmented eggs or isolated blastomeres. This possibility obviously depends on the arrangement in the cytoplasm of the various necessary materials, which must be such that a fragment or blastomere can receive a portion of each. It decreases with time in both cases, though for different reasons. The totipotence of egg-fragments diminishes apparently because there is a redistribution of the materials during maturation and fertilization, when for instance a bilateral replaces a previously radial symmetry, but isolated blastomeres cease to be able to give rise to whole embryos or larvae so soon as the cell-divisions fall in such a way as to divide the cytoplasm into unlike portions. Where the cell-plasma is highly differentiated this occurs at once, as in the bilateral eggs of Cynthia and the Ctenophores and usually in Molluscs: where the structure is polar, but not markedly bilateral, cells separated by meridional divisions are totipotent, those produced by equatorial or latitudinal divisions less so or not at all (Sea-urchins, Nemertines, Amphioxus). The case of *Ascaris* is very instructive: normally the first division is equatorial, but in the centrifuged egg meridional: in the former the half-blastomeres develop partially, in the latter totally. The egg of *Cerebratulus* provides a similar example; for the second furrow, which is normally meridional, may be made equatorial by pressure at right angles to the egg-axis (Yatsu). The quarter-blastomeres which in the first case are totipotent, are now no longer so, since two are animal and two vegetative. Lastly, where the egg-structure is radially symmetrical about the centre (as in Hydromedusae, with the exception of *Carmarina*), the cells remain totipotent as long as the divisions are perpendicular to the surface, but when tangential divisions occur by which the endoderm is delaminated from the ectoderm, this capacity is lost; at least it is known that these two germ-layers cannot

replace one another in regeneration. The power of giving rise to whole structures therefore only disappears because the cleavage takes a certain course, and, were the divisions to continue in one kind of direction, might conceivably be indefinitely retained, just as the compressed egg of *Nereis*, or the egg of *Cerebratulus* in calcium-free sea-water produces octettes instead of quartettes of micromeres.

The limitation of potentialities seen in later stages is due again to the original regional differences in the egg-material. Thus endoderm and ectoderm can as little replace one another in a Sea-urchin gastrula as in a regenerating Hydra, or in a Hydra that has been turned inside out (the experiment of the Abbé Trembley is well known). It is stated indeed (Driesch) that any fragment of a blastula of a Sea-urchin can give rise to as normal a larva as any other, but this needs re-examination.

On the other hand, organs normally formed by one part of the embryo can be developed by another. The posterior half-gastrula of a Nemertine develops an apical organ, that of a Sea-urchin an apical organ, a stomodaeum, and a tripartite gut; and here we are in the presence of the phenomenon which is characteristic of all regeneration, the development of a whole structure from a part of a differentiated structure.

When a (meridional) half- or quarter-blastomere of a polar egg is differentiated into a complete larva, that appears to be due to its possessing a share of each necessary substance; but when a worm regenerates a head, or a newt the lens of the eye, or a crayfish a limb, this simple explanation will not avail. Regeneration is not within our present scope, but the behaviour of certain blastomeres when isolated, raises precisely the same question.

We have seen that in the Frog not only from the four vegetative but also from the four animal cells a blastopore and archenteron can be developed. Similarly in the Sea-urchin, not only a vegetative, but also an animal, blastomere can gastrulate, though the latter does so neither as well nor as frequently as the former. In other words, a structure can

be formed by a part of the germ which is not ordinarily devoted to that end. We must therefore assume provisionally some such disposition of the organ-forming materials in the germ as that suggested by Boveri in his 'stratification' hypothesis. According to this conception the substances in question are distributed through the cytoplasm, but with concentrations decreasing in opposite directions, which directions are, in polar eggs, along the axis. Thus a substance on which the formation of the ectoderm depends is assumed to be most concentrated at the animal pole, less in the equator, and least at the vegetative pole, while there is a similar decrease of endoderm-forming substance in the reverse direction along the axis. Both animal and vegetative cells therefore possess the requisite materials for developing these structures, though not in the same proportions as in the entire ovum, nor as one another. Hence the differences in their capacities.

Such a stratification can of course in some cases be actually observed, for instance in the Amphibian egg, where yolk and cytoplasm are graded in opposite directions in this way, and even where not easily visible may be made apparent by the use of the centrifuge, since the several constituents of the cytoplasm are frequently of different specific gravities.

The effect of separating these constituents in this way has now been investigated on several eggs and much light thereby thrown on the significance of each one in development.

Ever since Born's cytological examination of the Frog's egg forcibly inverted in Pflüger's original experiment, it has been known that the heavy yolk-granules sank in the viscid cytoplasm while the lighter plasma rose to replace them, so conferring a new polarity upon the egg; and ever since Hertwig's experiment it has been known that by this use of the centrifuge the yolk could be driven much more rapidly to one side of the egg than under the influence of gravity alone. Such eggs frequently develop abnormally, with meroblastic segmentation, spina bifida of the embryo, and so on. The development of such centrifuged eggs has been more recently studied by Konopacka. The eggs, placed on slides in water, are centri-

fuged either slowly for a long time ($f = 10\,g$ to $12\,g$) or rapidly for a short time ($f = 228\,g$), and various stages are used, namely, unfertilized eggs, fertilized but unsegmented eggs, two-celled stages and eight-celled stages.

1. The eggs are centrifuged while still in the oviduct (presumably during the second maturation division), then removed and fertilized.

A. Slowly for five hours. The direction of the centrifugal force makes any angle with the egg-axis, and some eggs have the white pole uppermost. The first furrow appears at the normal time, but is often not meridional, the blastomeres being unequal. While normal embryos may be developed, the blastopore sometimes fails to close, and in a few cases only half the egg is segmented and develops into a half-embryo.

B. Rapidly for thirty minutes. At the centripetal pole—which may or may not be the original animal pole—there is an extrusion of hyaloplasm. The development of these eggs is as in the previous experiment.

It is to be noted that in these eggs the axis of centrifuging may make any angle with the original axis, that by the redistribution of yolk and cytoplasm a new polarity may therefore be conferred on the egg, and that the symmetry of the embryo is related to this new and not to the original polarity, the anterior end being developed at the centripetal pole, in precisely the same way as in Pflüger's forcibly inverted eggs.

2. Since the perivitelline fluid has been exuded the egg is free to turn inside the jelly membrane, and places itself upon the machine with its yolk-pole outwards. The egg is centrifuged, from fifteen minutes to one and a half hours after insemination.

A. Slowly for five hours. The results are very much the same as before; open blastopores and half-embryos occur.

B. Rapidly—i. Fifteen minutes after insemination for from ten minutes to thirty minutes. Three layers or strata appear, at right angles to the egg-axis (which coincides with the axis of centrifuging); at the animal (centripetal) pole is a layer

of yellow hyaloplasm, next this a layer of pigment, and finally the unchanged yolk may be seen to come to the surface at the vegetative (centrifugal) pole.

The embryos have depigmented heads, or are headless altogether, the anterior end being occupied by a swollen vesicle. The degree of deformity depends on the length of exposure to the centrifugal force.

ii. When the eggs are centrifuged one and a half to three hours after insemination for fifteen minutes or longer a fourth white stratum appears, between the yellow hyaloplasm and the pigment. The first division is usually unequal, and the small blastomere segments more rapidly than the large one. Many eggs die before gastrulation. Amongst those that do develop half-embryos and headless monsters are frequent.

3. The egg is centrifuged in the two-celled stage.

A. Slowly for five hours. The first division is unequal, and there is a tendency to meroblastic segmentation.

B. Rapidly for from five to twenty-five minutes. The same strata appear in each blastomere as in the second experiment, and, with the longer exposures, there are abnormalities of development, such as meroblastic segmentation.

4. The eggs and anterior half-embryos are centrifuged rapidly. After the completion of the third furrow the strata appear in each of the eight cells. There is a tendency to meroblastic segmentation, open blastopores, and anterior half-embryos. These experiments make it perfectly clear that derangement of the substances of the cytoplasm involves abnormality in development. In the first place the yolk is driven to the vegetative pole, and the distinction between the protoplasmic and deutoplasmic regions of the egg thereby increased. The yolk fails to segment, but the cytoplasm of the animal hemisphere divides, and gives rise to a blastoderm. The displacement of the yolk entails later on malformation of the posterior end, normally developed near the vegetative pole, with consequent persistence of the blastopore and restriction of differentiation to the anterior half of the embryo. The heads of these embryos are depigmented and the pigment is therefore presumably inessential, but when the derangement

is more serious, possibly because some other material is driven from the animal pole into the interior of the egg, there is no head at all.

Hertwig and Wetzel have studied the effect of centrifugal force upon the unfertilized egg.

Similar experiments have been performed on other ova. We turn first to those, carried out by Lyon, Morgan, and Spooner upon the eggs of the Sea-urchin *Arbacia,* in which there is a diffuse red pigment.

If the ripe but unfertilized ovum be strongly centrifuged ($f = 6400\,g$) four strata appear. The pigment passes to the centrifugal pole, next to this is a grey granular layer, blackened by osmic acid, then a fluid hyaline layer in which lies the nucleus, while the centripetal pole is occupied by a cap of opaque white material. The new axis of stratification which is thus produced by the operation may make any angle with the original axis as determined by the micropyle.

When removed from the centrifuge the strata begin to remingle, but the first and fourth return to their original positions very slowly if at all. The second and third layers on the other hand intermingle with one another rapidly, and it is apparently necessary that they should do so before segmentation and development can occur; for if the egg be broken into two portions between them, then neither portion can be fertilized.

In segmentation it is the axis of stratification which determines the direction of the furrows, since the first three, which are at right angles to one another as in the normal egg, either include or are at right angles to this axis, or, the axis of stratification coincides with one line of intersection between some two of these three divisions. At the next division the micromeres are formed at that intersection of two furrows which is at the anti-micropylar pole or nearest to it (when the axis of stratification is oblique to the original egg-axis).

It appears therefore that some invisible polarity of the egg has remained unaffected by the centrifugal force, and this

determines the symmetry of the embryo, since the micromere pole becomes the blastopore pole, and the original egg axis the gastrula axis, or as nearly so as possible. The pigment is found in any part of the larva, right or left, dorsal or ventral, anterior or posterior. It is not therefore essential to development. It may be added that the yellow pigment band of *Strongylocentrotus* is equally unnecessary. Normally it is subequatorial and passes into the archenteron, but it may be meridional or oblique to the egg-axis, and so become incorporated wholly or partly in the ectoderm (Garbowski).

Experiments of a like kind on other eggs have yielded like results; for while the existence of an invisible structure has been revealed, a structure which is not disturbed by the centrifuge and is definitely related to the subsequent differentiation, that differentiation has been shown to be independent of the distribution of some at least of the visible constituents of the cytoplasm.

Thus Lillie, by centrifuging the egg of *Chaetopterus* during the first maturation division, produced in it three layers: a small grey cap at the centripetal pole, a clear layer, and a yellow granular hemisphere (on the centrifugal side). These strata, it was found, might occupy any position with regard to the egg-axis (as defined by the polar bodies), yet in fertilization the sperm always entered at the vegetative pole, and cleavage was always normally related to that axis. The grey cap is derived from the contents of the germinal vesicle, the clear band from the microsomes of the endoplasm, and the yellow granules from the coarser endoplasmic constituents. It would be interesting to know the further history of these centrifuged eggs.

This we do know in other cases.

The ovum of the Lamellibranch *Cumingia* contains a red pigment and an oily green material both scattered through the cytoplasm. When the egg is centrifuged during the first polar division (Morgan) these go to opposite poles, the red pigment to the centrifugal, the green oil to the centripetal. Between the two is a broad hyaline layer. Maturation proceeds and the polar bodies are extruded.

With the egg-axis, as so determined, the axis of stratification may make any angle. Fertilization occurs, and in the subsequent cleavage the planes of division bear the normal relation to the axis of the egg. The strata persist, so that the red pigment may be in *AB* or *CD* and so on, the green oil on the opposite side. Development follows and these two coloured materials are found opposite to one another in any position in the trochophore larva, the structure of which has the normal relation to the cleavage system. There does, however, appear to be some tendency for the green oil to redistribute itself.

So again in Pulmonate eggs (*Physa*, *Planorbis*, *Limnaea*) Conklin has, by the same means, produced three strata: a grey finely granular zone at the centripetal pole, a narrower clear zone, and a yellow granular centrifugal hemisphere. When segmentation and development take place the strata make any angle with regard to the first and subsequent furrows, and any angle with the principal planes of the embryo. Conklin has, however, added the important observation that the possibility of obtaining a normal development is largely dependent on the redistribution of some of the disturbed cytoplasmic materials, for it is only when the operation is performed prior to maturation or during its earlier stages—only, that is, when some time elapses between the operation and cleavage—that development is afterwards normal. Eggs centrifuged during the extrusion of the first polar body, or later, either die or give rise to monstrous embryos. It appears further that during the interval, the clear substance disappears into the grey or the yellow layer or both, a readjustment which cannot occur unless sufficient time be allowed. In another Mollusc, *Crepidula*, on the other hand, and in the Ascidian *Cynthia*, Conklin has found it possible, by prolonged centrifuging, to shift the original polar axis (which in the experiments just quoted is left unaltered) without prejudice to normal development. The symmetry of cleavage and of differentiation are, it seems, determined by the new polarity as in the Frog's egg.

In *Cyclops* (Spooner) the centrifuge separates the cytoplasm

into three similar zones: a greenish-white layer at the centripetal pole, a middle clear stratum, and the blue yolk-granules. These eggs develop normally even when continuously centrifuged.

In the Rotifer *Hydatina* (Whitney) the polar zones are pink and grey, the middle clear as in the foregoing instances. The stratification may have any relation to the original axis; the first cleavage is, as in the normal egg, transverse to this axis, and normal young are produced, become mature, and reproduce in their turn.

Lastly, in the centrifuged egg of *Ascaris* similar strata appear. The normal egg, as pointed out already, is telolecithal. There are in the cytoplasm also some clear spherules and pigment granules. The layers that appear after centrifuging are a layer of yolk at the centripetal end (the yolk is here lighter than the cytoplasm), a layer of clear spherules, protoplasm, and finally, at the centrifugal pole, a cap of brown pigment. When strongly centrifuged, the egg becomes flattened against the slide on which it is placed (the centrifugal force is perpendicular to the slide), and, if still subjected to the action of the force, the fertilization spindle places itself at right angles to the direction of the force, that is, parallel to the stratification (and in the clear zone) and the division is meridional. If, on the other hand, the egg is removed from the machine it resumes its spherical shape and the spindle returns, more or less completely, to its proper axial position and the first division is equatorial (or oblique). It is suggested by Boveri and Miss Hogue (to whom the experiments are due) that there is an invisible polarized structure in the cytoplasm which is not affected by the operation, and with the axis of which the stratification of the movable substances can make any angle. Into the axis of this invisible polarity the spindle is supposed to return, if and when the egg is allowed to resume its spherical shape. The facts do not appear to necessitate this view, for when placed on the machine the whole egg rotates inside its shell until the heavier animal pole is centrifugal and then the stratification of the cytoplasmic materials begins. As long as the force is

operating, the spindle is compelled to place itself parallel to the stratification, but when released from the force, returns or attempts to return to its normal position, namely, in the egg (i.e. in the stratification) axis. The obliquity of the spindle, in those cases where the return to the normal position is not complete, would then be the result of two tendencies at right angles to one another, the one urging the spindle to place itself perpendicular, the other parallel, to the stratification.

When the spindle returns more or less completely to its normal situation, the division is equatorial or oblique and a normal embryo is developed in spite of the stratification. When, however, the spindle remains in the stratification plane, the first division is meridional and each cell behaves as the P_1 (vegetative) cell of an entire ovum. The greater part of the pigment zone is usually extruded from these eggs at the centrifugal pole, as a 'ball'. Each half-blastomere divides into two, which can be recognized as *E M St* and P_2 by the chromosomes being diminished in the one and intact in the other, and by their subsequent behaviour, and so gives rise to what is essentially a blastula without ectoderm (see Fig. 5, p. 13). It might be imagined that the ectodermal material had been extruded with the 'ball', but apparently this is not so since the development is the same when (as may happen) no ball is extruded.

It must be admitted that it is at the moment very difficult to build any very definite hypothesis upon the results of these various experiments with the centrifuge. It is obvious that by the rearrangement of some at least of the constituents of the cytoplasm a stratified polar structure may be easily imposed upon the egg, and that it is certainly not necessary that all of these materials should return to their original positions in order that development may be normal. The evidence does, however, indicate here and there (the Frog, the Sea-urchin, the Pulmonate) that time must be allowed for a recovery of some kind before development can be normal. The axis of the new polarity may entirely replace the original axis in the determination of embryonic symmetry (as in the

Frog) but certainly does not in other cases, where it appears that the old axis persists unaffected by the operation, not marked by any visible differentiation of materials, but causally related nevertheless to the symmetry of cleavage and development. That this axis has not been affected by the centrifuge hitherto does not, however, justify the assumption that it cannot be, and indeed Conklin has succeeded in shifting it in *Crepidula* and *Cynthia.* The polar structure to which it belongs may, therefore, eventually prove to be dependent on 'the heteropolar arrangement of certain oöplasmic substances', though these are indistinguishable to the eye, and need not of course be of sufficiently different specific gravities to allow the force applied to overcome their viscosities.

Taking all the results together, it seems that since it has been demonstrated that the different regions of the cytoplasm do play definite but different parts in development, are in fact determinants of characters which form an integral portion of the total inheritance of the species, the time is ripe for the physical and chemical investigation of the properties of the egg-plasma.

There remains for discussion Boveri's description of the development of dispermic eggs in *Ascaris*, the history of which shows clearly the influence upon the nucleus of the different regions of the cytoplasm.

These doubly fertilized eggs divide simultaneously into four cells, arranged in a tetrahedron.

The subsequent cleavage is of either one of three types according as there are one, two, or three P_1 cells, each at the end of a T-piece (Fig. 5, p. 13).

I. One of the four cells divides so that of its two products the outer touches its sister cell but no other cell in the germ. This cell is P_1 and divides into $EMSt$ and P_2. The three remaining cells are together equivalent to the AB of normal development and give rise to ectoderm. The somatic cells are recognized of course by the diminution of their chromatin, while the germ-cells have whole chromosomes.

II. Of the four cells two behave as P_1, two as S, and there

are therefore, after the next division, two figures of T, each of which continues to segment in every respect like a whole egg.

III. This type occurs frequently in centrifuged eggs. Of the four cells one is large and yolkless (animal), and behaves as S_1; while the remaining three are small and full of yolk, and each develops as a P_1.

The disperm eggs continue to segment, but give rise to irregular cell masses incapable of development beyond the gastrular stage, and that only in type I. Two primordial germ-cells were found in these gastrulae.

The number of chromosomes in these eggs is of course $3\,n$. There are four centres between which spindles are developed in various ways to form a quadripolar figure, the chromosomes being irregularly distributed on the equators of these. There they divide, and their halves are pulled in the ordinary way to the spindle-poles. The four cells may and do receive, therefore, different shares of the available $6\,n$, i. e. twelve chromosomes. Now in the normal egg there are always $2\,n$ chromosomes that remain intact, in each cell in the germ-track. If, therefore, the diminution were an intrinsic property of certain chromosomes (namely, the somatic) while conversely the intact chromosomes remained so by virtue of some internal cause, then there should be in a dispermic egg exactly six intact, and six diminished, chromosomes, and these would be irregularly distributed over the four cells and their descendants. The reverse of this is the case. In certain cells, the number of which differs in the three types, the chromosomes remain entire, whatever their number may be (it may be any number from two to twelve), while in the other cells the chromosomes are diminished, whatever their number. We know further that the diminution occurs in the dispermic, as in the normal egg, in the cells of the animal region, while in the vegetative cell or cells it is absent. It is necessary therefore to suppose that it is some difference in the cytoplasm of the animal hemisphere which causes the diminution.

We shall see in the next section that while there is evidence

that the n chromosomes of the germ-cells are unlike one another, yet the entire set contributed by each germ-cell is handed on to every cell in the body, and indeed must be if development is to be normal. The experiment which has just been described suggests that the cytoplasm, different in the various cells of the body, might incite to activity different elements of the chromatin in each case.

B. The Nucleus.

The experiments we have so far considered have shown us that while the nucleus is not qualitatively divided during segmentation and subsequent development, the cytoplasm, far from being isotropic, is heterogeneous, and its several parts causally related to the differentiation of certain elementary organs.

It does not, however, follow for a moment that the nucleus has no rôle to play in the process of differentiation, and there are indeed very sound reasons for believing that it is a vehicle of inheritable characters.

1. For in the first place experiments on Protozoa have proved that the nucleus, though not essential for irritability and locomotion, nor even for the ingestion of food, is yet necessary for the functions of metabolism and reproduction.

An enucleate piece of an *Amoeba* can ingest and even partially digest food, but the products of digestion cannot be assimilated. Any portion of an Infusorian (that is not too small), provided it contains a piece of the nucleus, can replace those parts of the whole structure which it lacks, can reproduce the original form.

2. Secondly, the study of the maturation of the germ-cells has shown that these elements, while unlike in every other respect, are yet identical in the number and size of the chromosomes of their nuclei (with the exception only of the heterochromosomes or sex chromosomes in Insects and others). Similar characters being inheritable from either parent, the determinants of such characters have naturally been imagined to reside in the nuclei, that is, in the chromosomes of the

germ-cells. Moreover, in fertilization the acrosome, sperm-head or nucleus, and centrosome, alone are essential, for the tail may be left outside. The functions of the acrosome—ensuring the entrance of the spermatozoon—and of the centrosome—making the sperm-sphere—are known: hence the nucleus is the seat of the determinants of those characters that can be transmitted by the male, and these are similar to those transmissible by the nucleus of the female. At the same time we know from the phenomena of merogony and artificial parthenogenesis that both nuclei are not necessary, but that one set of n chromosomes will suffice.

We have now to consider the evidence for believing that these n chromosomes are really different from one another.

3. Roux long ago pointed out that karyokinesis looked like an apparatus for simultaneously dividing and distributing to two cells a number of qualitatively unlike bodies, but the experimental proof of the dissimilarity has only recently been brought forward by Boveri.

This proof is based on the behaviour of dispermic eggs of the Sea-urchin *Strongylocentrotus lividus.*

The dispermy is caused, not by treatment with a poison (as in the experiments of the brothers Hertwig), but merely by adding a large quantity of sperm to the ova.

Of these dispermic eggs the following types may be distinguished:

I. The tetraster (each sperm produces a centrosome which divides), followed by simultaneous quadripartition. There are spindles between all four centres.

a. Plane tetraster. The four cells lie in one plane, parallel to the equator of the egg.

Another meridional division gives eight cells in a ring. An equatorial cleavage separates animal from vegetative blastomeres, and there follows the formation of sixteen mesomeres, eight macromeres, and eight micromeres.

b. Tetrahedral tetraster.

There are never eight micromeres and macromeres, but either six or four of each.

II. The double spindle.

One sperm nucleus with its two centres and the spindle between them remains apart from the other. The latter conjugates with the female pronucleus.

a. Both spindles lie in the same plane.

b. They are at right angles to one another, that is tetrahedrally placed.

These eggs usually divide into two, occasionally (Teichmann) into four. On one side of the egg are nuclei with $2n$, on the other nuclei with n chromosomes. (These double-spindle eggs

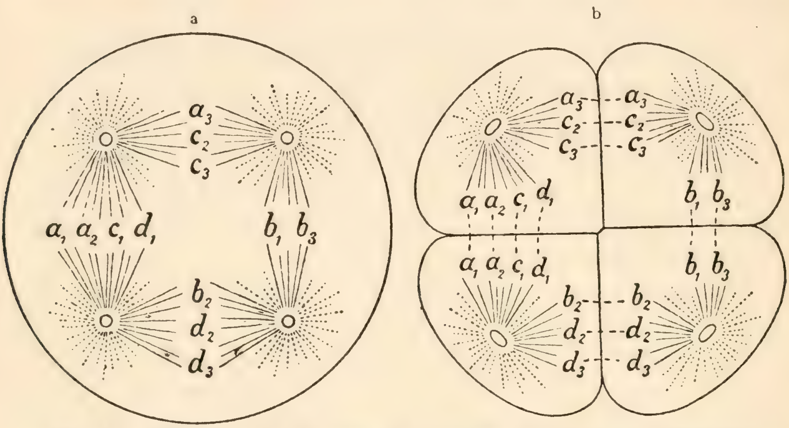


FIG. 17.—Diagram of one case of irregular chromosome distribution in a doubly fertilized egg ; a_1, b_1, c_1, and d_1 ; a_2, b_2, c_2, and d_2, and a_3, b_3, c_3, and d_3 are the three complete, specific sets of unlike chromosomes. (After Boveri, 1904.)

have been referred to already in another connexion. To our present purpose they are irrelevant.)

III. The triaster.

By shaking the eggs the division of one centre is prevented.

a. Triaster proper, with spindles between all three centres.

b. Amphiaster-monaster, in which one centre remains apart.

The triaster divides simultaneously into three by meridional divisions, and then into six by another meridional cleavage. An equatorial division is followed by the production of twelve mesomeres, six macromeres, and six micromeres.

IV. Both centres may fail to divide.

a. An amphiaster is formed between them.

b. They remain apart.

The first and third types are those which immediately interest us. In these tetracentric and tricentric, simultaneously quadripartite and tripartite ova, the $3n$ chromosomes divide and the $6n$ chromosomes are then thrown at random on the equators of the spindles connecting the various centres. In the anaphase of the mitosis the daughter chromosomes pass to the four or three centres, and the four or three cells consequently receive a random number of the elements. Assuming for the moment that the chromosomes are qualitatively unlike, each cell also receives a perfectly random assortment of them, and the chance of every cell receiving at least one of each kind of chromosome is very small indeed, but greater of course for the tripartite than for the quadripartite ova. The chance, however, that one cell will receive a full complement is much larger.

A study of the development shows that the quadripartite practically never develop normally, the tripartite sometimes but not often, while, if the four cells of the former or the three cells of the latter be isolated and allowed to develop independently, a very fair percentage of normal larvae is obtained. All four, or three, develop differently. In the first case one or even two may give normal larvae, but never all four, while of the three cells of the triasters one or two, rarely all three, reach the pluteus stage.

The same differences between the blastomeres are seen when they develop in connexion with one another.

The tripartite ova gave a mean of 8% normal larvae, out of 828 reared in all.

The normal larva consists of three regions, marked by the different size of the nuclei, dependent on the different number of chromosomes in the original three cells. The egg-axis, segmentation-axis, and gastrula-axis being all coincident, the boundaries between the three regions meet in the blastopore at one end, the animal pole (anterior end) at the other. One boundary is generally in the median longitudinal plane.

Special cases are (1) where the nuclei are not of different sizes and the regions therefore indistinguishable; (2) where

the dimensions (surfaces) of the nuclei are as 1 : 2 : 3; this is accounted for by supposing only two of the centres to have been united by spindles (this was actually observed) one of which was occupied by one sperm-nucleus, the other by the second male and the female nucleus: on division the centres would receive only $2n$ and $3n$ chromosomes. (3) By supposing the numbers of chromosomes on the three spindles to have been $\frac{n}{2}$, $\frac{n}{2}$ and $2n$, the centres, on the division of these, would receive n, $2\frac{1}{2}n$, and $2\frac{1}{2}n$. This would account for the ratios of the dimensions of the nuclei observed in the three regions.

Lastly, amongst these larvae are asymmetrical forms. It is suggested that this is due to some slight difference between the sperms, such differences having been in fact observed amongst larvae of the same monosperm culture.

In the remainder of the embryos or larvae reared from tripartite ova, more or less serious defects were found. Thus the skeleton is incomplete or wanting in one part, the pigment-cells may be entirely absent from some one region, but never from the same part. The defective region is always characterized also by the size of its nuclei, that is, is derived from one of the original three cells. In other cases one-third, two-thirds, or all three are pathological, that is, break up into cells which pass into the blastocoel and there degenerate. The remainder (if two-thirds) may develop into a normal larva if the pathological one-third has been got rid of at an early stage, but if not till later, then the two-thirds larva shows irregularities in skeleton. These irregularities are supposed to depend on (1) the relation of the triaster to the (assumed) plane of bilateral symmetry in the ovum, and (2) the position of the degenerate cell, whether dorsal, ventral, right, left, anterolateral, or posterolateral. The nuclei in the two regions of the sound part may be of different sizes.

When two-thirds are degenerate only stereoblastulae or stereogastrulae are produced.

Of 1,600 quadripartite ova kept under observation, only thirteen reached the pluteus stage, and only three of these

really deserved the name of pluteus. Of these three one had to be removed to the category of double-spindled eggs, another was probably a $\frac{3}{4}$ egg, while the third had an abnormal skeleton. In this last there were three sizes of nuclei, small in one quarter, medium in one quarter, and large in one half of the larva, and a supposed distribution of chromosomes in the tetraster is suggested to account for this.

In the remaining ten, which did not deserve the name and style of pluteus, there were disintegrated cells in the blastocoel, imagined to be derived from one of the four blastomeres, while three areas characterized by the different size of their nuclei, and each one-third of the whole, were found in the larva.

Usually, however, only one half or one quarter of the egg develops.

The abnormality of development is considered by Boveri to be directly due to the irregular distribution of chromosomes, which is such that the chance of each cell in a quadripartite tetraster egg receiving a full set at least of the n chromosomes is practically negligible. The chance of each cell of a tripartite triaster egg receiving a complete set is however appreciable, and a certain percentage of these develop normally. The chance of one cell in either case receiving the total complement is greater still, and these when isolated give rise to normal plutei in a fair proportion of the cases.

It is concluded, therefore, that the chromosomes are different, and that at least one complete set of the n chromosomes of the species is necessary, not merely in the ovum, but in every cell into which that ovum divides in order that its development may be normal.

Such alternative hypotheses, as that the abnormality observed is due merely to irregularities in the number of chromosomes, are negatived by the known normal development of plutei with nuclei of different sizes in the different regions of the body, the sizes being determined (see above) by the number of the chromosomes, and being such that the number may have been less than n, or greater than n, and in the latter case either a multiple of n or not.

The chromosomes are therefore different, and probably them-

selves made up of unlike elements, the elements which conjugate in the synapsis prior to the maturation divisions. For normal development of the whole and of each part a complete set must be present in every cell. In sexual reproduction two such sets are present, but one suffices.

To the evidence thus brought forward by Boveri must be added certain cytological observations on the different size and form of one or more of the chromosomes. Thus Sutton found that in the Insect *Brachystola* the chromosomes of the spermogonia could be arranged in n pairs according to their sizes, and that in the maturation divisions the members of the several pairs conjugated and then separated from one another. Baltzer again and Tennent have found straight, hook-shaped, and horse-shoe shaped chromosomes in Echinids, while Wilson and others have demonstrated the heterochromosomes in many Insects and possibly some other animals.

In what way these various chromatic elements call forth the development of those characters which they transmit is unknown, but it seems certain that the differential activity of nuclei which are alike must depend on differences in the environment in which they are placed, that is, on differences in the cytoplasm. Such we know to exist, and we know also that they can provoke dissimilar behaviour in similar nuclei. We are therefore brought back to the structure of the cytoplasm as a necessary condition of the transmission of characters by the nuclei.

It remains for us to consider exactly what kind of characters are handed on by the plasma directly and by the nuclei. Experiments on heterogeneous hybridization enable us to give at least a provisional answer to this question.

The possibility of fertilizing an ovum with the spermatozoon of an animal of quite a different kind was the discovery of Loeb, who found that by the addition of a small quantity of calcium chloride and sodium hydrate to sea-water, the eggs of the Sea-urchin *Strongylocentrotus purpuratus* would permit the entrance of spermatozoa of the Starfish *Asterias ochracea*. From these eggs pluteus larvae with the charac-

teristic skeleton are developed. The starfish larva is of course the Bipinnaria, without skeleton. The problem was taken up next by Godlewski, who used the Crinoid *Antedon* as the male parent, the Sea-urchins *Sphaerechinus, Echinus*, and *Strongylocentrotus* as the female. The sexual elements must first be treated with hyperalkaline sea-water.

Fertilization is perfectly normal with production of a vitelline membrane, rotation of the sperm-head, union of the male and female pronuclei, and so forth. Cleavage is of the Echinoid type with formation of micromeres at the fourth division; no such micromeres occur in *Antedon*, where the fourth cleavage is meridional in both hemispheres. Primary mesenchyme (absent in *Antedon*) is formed, gastrulation follows, and then a typical pluteus with the characteristic skeleton is developed. The larva of *Antedon* has no skeleton. The *Antedon* chromosomes persist, and the nuclei of the hybrids are intermediate in size between those of the parent forms. Baltzer, who has repeated the experiment, confirms this as well as all other details.

Enucleate egg-fragments of *Echinus* were also fertilized by Godlewski with *Antedon* sperm. The details of fertilization were normal, but segmentation was irregular, and after gastrulation development ceased. Primary mesenchyme was differentiated and the archenteron inclined to the oral side, both Echinoid characters.

In spite, therefore, of the persistence of the male chromosomes the larva is of the pure maternal type.

The Sea-urchin egg may also be fertilized by the spermatozoa of Molluscs and Worms.

Loeb, employing the Mollusc *Chlorostoma*, found that a membrane was extruded, that cleavage was normal in form and rate, and that normal plutei were developed. The cytology was not investigated.

A more extensive series of experiments is due to Kupelwieser. If the egg of *Strongylocentrotus* or *Echinus* be fertilized with the sperm of the Mussel *Mytilus*, no membrane is formed and polyspermy is frequent. The sperm-nucleus having rotated, preceded by its sphere, moves towards the egg-nucleus.

Segmentation is irregular, but the swimming blastulae become spherical and give rise to normal plutei, at the normal rate.

Though the male nucleus unites without fusing with the female nucleus, in the spindle developed between the two sperm-centrosomes it does not break up into chromosomes, and eventually passes undivided to one pole and into one cell. The female chromosomes divide in the usual way. The sperm-nucleus degenerates ultimately. It may not even have approached the female nucleus in the earlier stages, but have remained apart from the beginning.

The same author has found that membrane formation can be incited in the Sea-urchin (*Echinus*) egg by spermatozoa of a number of Molluscs (*Lithodomus, Mactra, Modiolaria, Pecten, Venus, Patella, Gibbula, Murex, Nassa, Trochus, Fusus*) and by those of the Polychaets *Aricia* and *Audouinia*. Only when *Mytilus, Mactra*, *Patella*, *Aricia*, and *Audouinia* are used does development follow. It has been shown elsewhere that membrane formation and the incitement to develop are two independent phases of fertilization.

For ensuring fertilization with *Audouinia* sperm treatment with alkali is unnecessary. The sperm enters, the head swells up, and a centrosome and aster are developed. The male nucleus may then unite more or less completely with the female nucleus, or remain apart. In the latter case it passes into one blastomere and degenerates, as does the *Mytilus* sperm in the *Strongylocentrotus* egg. In the former case when the spindle is developed and the female chromosomes are placed upon it, the sperm chromatin is seen in the form not of chromosomes but of one or more irregular lumps lying outside, on, or inside, the spindle.

When in the anaphase the female chromosomes become vesicular, the male lumps of chromatin do the same; they are distributed sometimes to both blastomeres, sometimes to one only. In the blastomeres the sperm chromatin may unite with the (female) nuclei but need not do so, and there is always a tendency for it to become gradually eliminated, for it is found in fewer and fewer cells as development proceeds, and the nuclei are found to be of half the size (surface) of the

amphikaryotic nuclei of normally fertilized ova. The irregularity in the behaviour of the male chromatin sometimes affects the female, and one or more of the cells may fail to obtain a complete set, and this is put forward as the cause of the irregular development of some of the larvae.

The segmentation of these ova is of the usual Echinoid type (in a certain percentage of cases) and swimming blastulae, and later, plutei, are produced from them. The rate is slow.

Godlewski has found that when Echinoid eggs are fertilized with the sperm of *Chaetopterus* or *Dentalium* the elimination of what is presumably the male chromatin takes place after the male and female pronuclei have fused, but before the chromosomes and the spindle appear.

In all these cases the larva is of the purely maternal type in every respect; it is a pluteus, and shows no trace of any of the characters of the larva of the male parental form. The earlier processes of cleavage and gastrulation are also those characteristic of the ovum employed. The male chromosomes may persist or be sooner or later eliminated. In no case do they exert the slightest influence.

Identical results have been obtained by Moenkhaus and Loeb with the heterogeneous hybridization of fishes. The egg of *Fundulus heteroclitus* is employed, while the distinct genera, *Menidia*, *Ctenolabrus*, and *Stenotomus* have served as the male parent. In fertilization the male (*Menidia*) chromosomes have been shown by Moenkhaus to be properly formed, and to persist in later stages. From the egg arises an embryo, which is however defective with cyclops eye, small head, pigment not distributed over the blood-vessels, and circulation not established though the heart is formed. The pigment-cells, red and black, are those characteristic of the female parent; red pigment not being present in any of the forms used as males. It appears, however, that the red pigment may be transmitted by the sperm, since in the reverse cross of Menidia ♀ × Fundulus ♂ it appeared in two cases.

It does not seem possible at this moment to say whether the total failure of the male characters to appear in these

heterogeneous hybrids is due to the inability of the male chromosomes to exert their activity in a cytoplasm to which they are not adapted, or to the fact that such characters as appear are not carried by the chromosomes at all. If the latter were true then it might be supposed—since the germ-cells of the two sexes each carry a complete set of the necessary specific chromosomes—that some at least of the characters of the larvae were not carried by the female chromosomes either, but simply by the cytoplasm, a supposition which is certainly strengthened by the appearance of certain Echinoid characters—the primary mesenchyme, and the inclination of the archenteron to the oral side—in the gastrulae reared from enucleate Sea-urchin egg-fragments fertilized with *Antedon* sperm. In addition to this there is the evidence from the defective development of ova from which certain parts of the cytoplasm have been removed.

While therefore we know that the determinants for some characters reside in the cytoplasm, the results of heterogeneous hybridization certainly suggest that these characters are the large ones, those that put the organism in its phylum, class, order, and family, the characters that make it an Echinoderm and not a Worm or Mollusc, a Sea-urchin and not a Starfish or a Feather-star, while the smaller characters, generic, specific, individual are transmitted by the nucleus. The former are perpetuated therefore by the female parent alone, the latter equally by both parents.

In the last resort, as we know, the cytoplasm is indebted to the nucleus for several contributions to its structure—the 'yolk-nucleus' and the contents of the germinal vesicle at maturation—but these are processes which find no counterpart during the formation of the male cells, except perhaps in the seemingly insignificant chromatoid accessory bodies.

It has been suggested (by Driesch) that the characters handed on through the cytoplasm are those that appear early in development, while those that arise later are carried by the nuclei. This is probably in the main true, as we have known, since von Baer taught us, that the general appears before the particular—'aus dem allgemeineren Typus bildet

sich also der speziellere hervor'. It is also in accord with the hypothesis put forward above that the various determinants comprised in the chromosomes will only be able to become active under the influence of different parts of the cytoplasm—only, that is, after the nuclei have been distributed during segmentation.

Further, early characters, such as the rate of cleavage, are known to be sometimes transmitted by the male cell as in hybrids of *Fundulus* (Newman); the rate may very well depend on the male centrosome, but that is of nuclear origin though not derived from the chromosomes.

It seems preferable therefore to base the distinction between the parts played by the two germ-cells in inheritance on the magnitude of the characters concerned.

The cytoplasm may of course carry some specific characters, such as the pigment of the egg and of the embryo, for instance in Amphibia; the cuticle, which may persist as that of the larva in Polychaets and the size of the egg, which may be correlated with that of the embryo, though this last is not necessarily so, since Conklin has found that the larger species of *Crepidula* usually have the smaller eggs.

It is to be observed that the characters which common experience and the experiments of breeders and those interested in inheritance suggest are transmitted by the male as well as by the female, and therefore through the intermediation of the nucleus, are just these smaller individual, varietal, specific and generic characters. The larger characters have always been tacitly omitted from consideration, for the simple reason that the investigation of them could only become possible by the discovery of some means of bringing about heterogeneous fertilization.

Into the evidence, based on common experience and scientific experiment, for the transmission of characters of some sort by the male parent, and therefore by the nucleus, it is quite unnecessary to go at length; but the hybrids between various genera of Sea-urchins have been such classical objects for investigations of this kind, ever since the days of Boveri's supposed production of a dwarf larva with the characters of

the male parent (*Echinus*) from a fertilized enucleate fragment of a *Sphaerechinus* egg, that brief mention may be made of the more recent researches.

It will be recalled that the plutei of the two genera *Echinus* and *Sphaerechinus* differ typically from one another, according to Boveri. The latter (Fig. 18) is short and squat, the oral lappet not divided into lobes, and the skeleton provided with a fenestrated anal arm—produced by three long parallel bars united by numerous cross-bars—an apical branch to the oral arm and, at the apex, a square 'frame' formed by the union of twigs from the last-mentioned and from the apical arms. The former (Fig. 19) is long and lank, the oral lappet is deeply

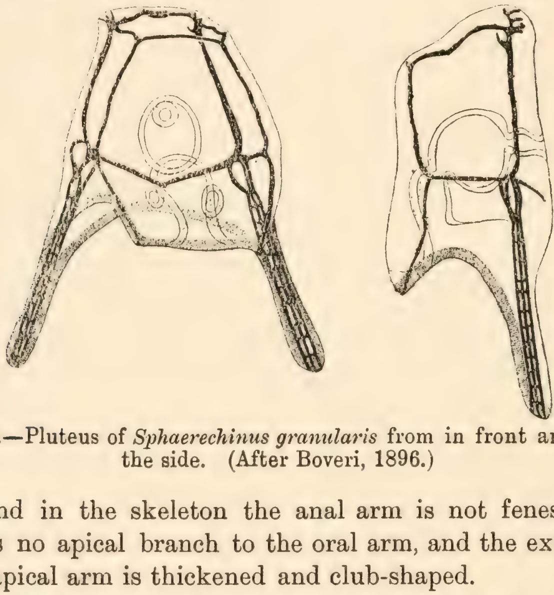


FIG. 18.—Pluteus of *Sphaerechinus granularis* from in front and from the side. (After Boveri, 1896.)

cleft, and in the skeleton the anal arm is not fenestrated; there is no apical branch to the oral arm, and the extremity of the apical arm is thickened and club-shaped.

The hybrid larvae (Fig. 20) were described by Boveri as being intermediate in form, shorter and broader than those of *Echinus*, longer and narrower than those of *Sphaerechinus*, and having the oral lappet slightly divided; while in the skeleton, the extremity of the apical arm was swollen and branched, the anal arms double but not fenestrated, and the oral arms sometimes provided with an apical branch.

Seeliger, however, pointed out that there is much variability

in the parental types—the extremity of the apical arm of *Echinus*, for instance, is not always club-shaped—and that in the hybrid cultures there are to be found, together with variable larvae of more or less intermediate type, individuals with purely paternal characters, though the pure *Sphaerechinus* type never occurs. This contention has been upheld by Morgan and by Steinbrück, the latter of whom has used *Strongylocentrotus*, the pluteus of which is hardly distinguishable from that of *Echinus*, as the male parent. Steinbrück

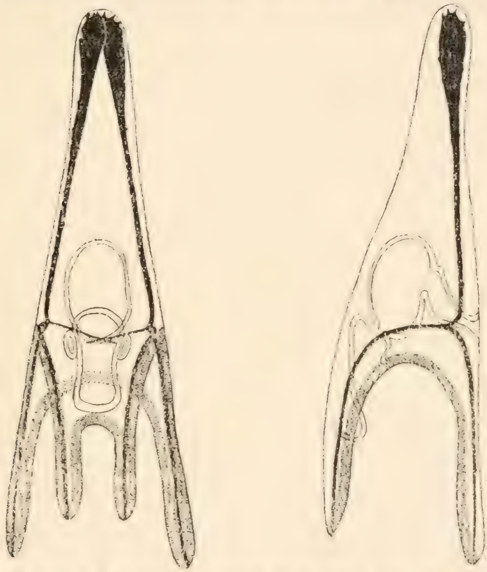


FIG. 19.—Pluteus of *Echinus microtuberculatus* from in front and from the side. (After Boveri, 1896.)

finds that the pluteus of *Strongylocentrotus* is variable (1) in the termination of the apical arm, (2) in the anal arm, which may fork, or be double or even treble throughout, though cross-bars are not seen, and (3) in the length of the oral and of the transverse arms. In the pluteus of *Sphaerechinus* (1) the apical 'frame' may be lacking, (2) the apical arms may fork and meet one another, but not be met by the oro-apical branches, and (3) the cross-bars of the anal arm may be few or absent. Most of the hybrids are intermediate, there is

usually an emargination between the oral arms, and the apex of the body is prismatic. In the skeleton the extremities of the apical arms are simple or branched and antler-like, but never club-shaped ; they may be wide apart, touching, or even fused ; the anal arm may be single, double, or treble, cross-pieces are rare ; the oro-apical branch is usually lacking, and seldom reaches the apical, and the transverse arms generally cross.

While, therefore, it is possible to say of a whole culture whether it is of the *Strongylocentrotus*, of the *Sphaerechinus*, or of the hybrid type, no such assertion can be made of an individual larva.

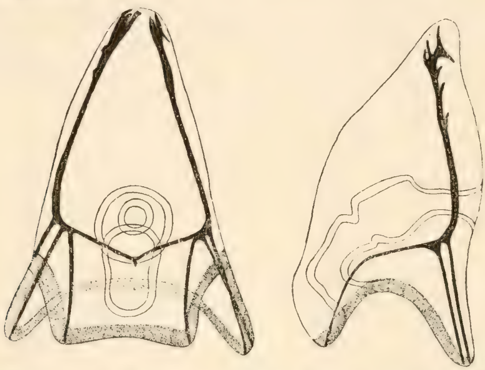


FIG. 20.—Pluteus of the cross *Sphaerechinus granularis* ♀ × *Echinus microtuberculatus* ♂, from in front and from the side. (After Boveri, 1896.)

Under these circumstances the difficulty of determining whether a given character is transmissible from the male parent is obvious. Vernon, however, using *Strongylocentrotus* for the male and *Sphaerechinus* for the female parent, found it possible to obtain a culture of purely paternal type in respect of the skeleton, in the winter, when the sexual maturity of the male parent is at a maximum. This is stated by Doncaster to be due merely to the lower temperature, and the same conclusion is reached by Herbst, who has examined the effect of temperature changes on the larvae of the pure parental forms and the hybrid larvae simultaneously.

The temperatures used were from 11°–19° and from 24°–27°. Thus in the hybrids the cross-bars of the anal arms are more

abundant at the higher temperature. This is an inheritance from the female parent (*Sphaerechinus*), in the pure type of which a rise of temperature has the same effect. Of the number of roots in the anal arms it is impossible to make a definite statement since heat increases these in both parents. The ratio of the length of the apical to the length of the anal arm is 1 in *Strongylocentrotus,* and greater than 1 at the higher temperature, 0·5 in *Sphaerechinus* and decreased at the higher temperature, while in the hybrids it approaches 0·5 at higher, 1 at lower temperatures; this appears therefore to be determined from the female side.

Other means have been used by Herbst to displace the inheritance to the female side, namely, the combination of artificial parthenogenesis with cross-fertilization. The egg is thus given an initial impetus towards the development of the maternal characters: the paternal are then superadded.

To this end the ova (of *Sphaerechinus*) are treated with butyric or other fatty acid, and subsequently with sea-water, in such a way that while the fertilization membrane is not thrown off, the nucleus yet enlarges and becomes indistinct, suggesting the passage of substances from it into the cytoplasm. After a suitable interval they are fertilized (by *Strongylocentrotus* sperm). Irregular segmentation follows and larvae are produced which have a greater similarity to larvae of the pure *Sphaerechinus* type than have the ordinary hybrids, though they are not completely of that type. The increase of maternal characters is evinced by the greater number of cross-bars in the anal arms, the greater number of roots to the anal arms, the greater length of the oro-apical branch, the branched extremity of the apical arm and by the decrease in the ratio of apical to anal arm (less than 0·5).

Cytological examination shows that the sperm always enters, rotates, and develops its sphere and centrosome. The female nucleus is in some cases only just resolving itself into chromosomes, in others this has occurred and the monaster has appeared.

In the first case the male and female nuclei unite and a fertilization spindle appears. On it are thrown both sets of

chromosomes, but the male are retarded and smaller, so that while the daughter female chromosomes are passing to the spindle-poles and becoming vesicular, the male are not so far advanced and still lie about on the spindle. They may divide, and their halves pass to the poles, but they often fail in this, and then either pass undivided to one pole or the other, or else get left behind to disintegrate in the cytoplasm. One cell may therefore easily fail to obtain a full set of male chromosomes, and the preponderance of female over male chromatin in one cell accounts for the smaller nuclei and preponderance of female over male characters on one side of the larva. This is incompletely partial thelykaryosis. Completely partial thelykaryosis also occurs—that is, all the male chromosomes go into one cell—and the larva has *Sphaerechinus* characters on one side, hybrid on the other, with small nuclei in the first and large in the second half.

In the second case the female nucleus has advanced as far as the monaster condition by the time that the spermatozoon enters—that is, the female chromosomes have divided and there are radiations about the female nucleus.

The female nucleus is now reconstituted from the chromosomes; the male nucleus approaches but does not as a rule unite with it.

The female monaster now degenerates, the $2n$ female chromosomes reappear and are thrown on the equator of the spindle formed in the meantime between the two sperm-centres. The male nucleus may (α) break up into chromosomes which lie either with or apart from the female chromosomes on the spindle, or (β) (and this is more usual) remain intact and lie on the spindle-equator, near one spindle-pole, or remotely in the cytoplasm.

Should male chromosomes have been formed they are dragged out as irregular strings and so divided (transversely); if not, then the nucleus is either divided amitotically, or else passes undivided into one blastomere. Except in the last contingency, the paternal chromatin must be irregularly distributed to the two blastomeres, and even when the nucleus does pass entire to one cell and fuse with the female

nucleus there it becomes irregularly divided in the next or some following cleavage. Partial thelykaryosis therefore never occurs. Hence also ultimately no cell in the body can have the full set of paternal chromosomes. Occasionally composite spindles are found in these eggs—that is, between the female monaster and an undivided male centre.

There seems little room for doubt, when all these results are considered, that the characters of the skeleton and body-shape at least, by which one genus of pluteus is distinguishable from another, are transmissible from the male as well as from the female parent, and therefore by the nucleus.

The experiments of others, while they lead to the like conclusion, illustrate also the difficulty of discovering the causes which determine whether the characters transmitted by the nuclei shall or shall not become manifest in the hybrid.

Thus MacBride, crossing *Echinocardium cordatum* ♀ with *Echinus esculentus* ♂, finds that the hybrid larva is smaller than that of either pure type, that the aboral spike characteristic of the skeleton of the pluteus of the female parent is absent, but that a male parental character, the bending in of the ends of the apical rods, is present, while the skeleton of the post-oral (anal) arms may be of the maternal, of the paternal or of an intermediate type.

Shearer, De Morgan, and Fuchs employ not the highly variable skeletal characters of the early plutei, but the stable features of the later larvae, such as the posterior pedicellaria found in *Echinus esculentus* and *acutus* but not in *Echinus miliaris*, the posterior epaulettes found in the two former but not in the latter, and the green pigment found in *miliaris* but not in the other two. When *esculentus* or *acutus* is crossed with *miliaris* the posterior epaulette appears, whether the parental form which possesses it is used as a male or female, while the green pigment of *miliaris* is not developed by the hybrid, whether this form has been used as male or female. This result, confirmed by Debaisieux, is not however invariable, for on previous occasions the authors referred to had found that the characters in question were always transmitted

through the ovum, never through the spermatozoon. The later results obtained by these authors and by Debaisieux suggest that, under certain conditions at least, the posterior epaulettes are dominant, the green pigment recessive, and a similar alternate inheritance of either a paternal or a maternal character, but in any case in its entirety, was reported by Loeb, King, and Moore in the hybrid *Strongylocentrotus franciscanus* ♂ × *Strongylocentrotus purpuratus* ♀. So Tennent has found that in the cross *Toxopneustes variegatus* × *Hipponoe esculenta* the characters of the latter are always dominant in the hybrid, whether it is used as the ♂ or ♀ parent. The dominance is not, however, absolute, since only about 70% of the plutei have the *Hipponoe* structure (*Sphaerechinus* type, while *Toxopneustes* has a larva like that of *Echinus*), and even then it is not pure.

Tennent makes the interesting observation that the dominance can be altered by decreasing the alkalinity of the sea-water. Under these conditions the percentage of hybrids of the *Hipponoe* type sinks to about 20%. Cytological investigation has shown that while in one cross (*Hipponoe* ♀ × *Toxopneustes* ♂) there is some elimination of chromosomes, though whether paternal or maternal is not known, there is no such elimination in the reciprocal cross.

Baltzer, who has studied the various crosses of *Arbacia pustulosa*, *Strongylocentrotus lividus*, *Echinus esculentus*, and *Sphaerechinus granularis*, finds that elimination of chromosomes occurs in certain cases only, and has further succeeded in identifying the eliminated chromosomes as paternal, and in showing that in these cases the skeleton of the hybrid pluteus is of the maternal type, whereas otherwise it is intermediate.

Thus the cross *Sphaerechinus* ♀ × *Strongylocentrotus* ♂ gives plutei with intermediate skeleton, as we know, but the plutei reared from the reciprocal cross are of the pure *Strongylocentrotus* type. The cytological examination shows that fertilization is normal up to the metaphase, but that then certain chromosomes begin to lag behind, and so are cast out instead of entering into the daughter nuclei. This elimina-

tion continues in later divisions until ultimately about sixteen or seventeen chromosomes in all are got rid of. The germ-number in *Strongylocentrotus* is eighteen, and among these is a long hook-shaped chromosome, which is always found in the hybrids; the germ-number in *Sphaerechinus* is twenty, and amongst these are two very long chromosomes; these are never found in the hybrids.

In the larva reared from the disperm egg there should therefore be 18 + 20 + 20 chromosomes, but the same elimination occurring, there are only twenty-six, while the monosperm larva has twenty-two. In other words, the disperm larva has lost thirty-two while the monosperm has lost sixteen, the disperm has retained 18 + 4 + 4, while the monosperm has retained 18 + 4. Hence it is the paternal chromosomes which have been eliminated, all but four. In the cross *Echinus* ♂ × *Sphaerechinus* ♀ there is no elimination of chromosomes, and the larva is intermediate. In the reciprocal cross the elimination occurs, but the paternity of the lost chromosomes has not been demonstrated, though it is known that the characteristic long hook and horse-shoe of *Echinus* remain.

In the cross *Strongylocentrotus* ♀ (or *Echinus* ♀) × *Arbacia* ♂, the larva is of the maternal type, and there is elimination of chromosomes. This, however, does not occur until the blastula stage is reached. It is not possible to be certain that the eliminated elements are paternal. In the reciprocal cross there is also elimination but the pluteus stage is never reached.

The discussions of the foregoing section may now be very shortly summarized.

1. Experiment shows that the removal of certain parts of the cytoplasm of the ovum entails the absence or at least the defective development of certain organs of the embryo or larva. Hence there are in the cytoplasm certain material factors on which the formation of certain characters depends. These characters are part of the total inheritance.

2. Every visible substance in the cytoplasm is not, however, necessarily such an organ-forming substance as experiments with the centrifuge demonstrate.

3. Experiments on heterogeneous hybridization indicate that it is the large characters—those of the phylum, class, order, family to which the animal belongs—that are carried by the cytoplasm, and, this means, transmitted through the female germ-cell alone.

4. At the same time the cytoplasm is, during pre-maturation stages, indebted to the nucleus for certain elements in its structure. In the female, therefore, these nuclear elements of the cytoplasm are concerned in the transmission of inheritable characters, as well as the chromosomes.

5. In the chromosomes, the germ-cells of the two sexes are alike, and these chromosomes are certainly concerned in the transmission of some characters.

6. It is known (α) by observation, (β) by experiment that there are qualitative differences between individual chromosomes, and that a complete set of these different chromosomes must be possessed by every cell in the body if development is to be normal. It is further probable that the chromosomes themselves are heterogeneous.

7. The different activities of the different chromatic elements are probably only called forth by differences in their environment, that is, in the cytoplasm to which they are distributed. It is known that differential behaviour of nuclei can be incited by cytoplasmic dissimilarity.

8. Hybridization experiments on nearly related forms make it certain that the smaller characters—generic, specific, varietal, and individual—can be transmitted as easily from the father as from the mother, and therefore through the nuclei.

II. The Interaction of the Parts upon One Another.

The evidence discussed so far has taught us that the undeveloped egg possesses a structure, both in its cytoplasm and in its nucleus, the parts of which are definitely related to the formation of certain organs of the embryo. It does not, however, follow that every separately inheritable character

of the animal is represented by a separate unit or determinant in the germ, whether in the cytoplasm or in the nucleus, and there is indeed evidence to show that certain characters are caused not by the independent development of any such determinant, but by the action upon one another of parts already in existence. The importance of this conception is obvious. The initial structure of the germ need not be unnecessarily complicated by imagining it to comprise an enormously large number of such separate material factors or determinants. Given a certain number to start with, the rest will follow by the actions and interactions of these upon one another. Such interactions may probably be of the nature, not of grossly mechanical influences, but of responses to stimuli.

Thus in the fish *Fundulus* the yolk-sac is deeply pigmented, the chromatophores being especially closely aggregated around the blood-vessels. When the creature is deprived of oxygen the pigment disappears, and it is supposed that under normal conditions the pigment-cells are chemotactically attracted by the oxygen in the blood (Loeb).

Again, Herbst has found that when the larvae of Sea-urchins are placed in solutions of lithium salts or in sea-water deprived of the sulph-ion, or devoid of the carbon-ion, the skeleton of the pluteus is either distorted in the first two cases, or absent in the last. The distortion takes the form of a multiplication of the tri-radiate spicules and the arms are correspondingly multiplied. In water devoid of CO_2 there are no spicules and no arms. Herbst has therefore urged that normally the outgrowth of the ciliated ring into the arms is due to a stimulus—thigmotropic perhaps—exerted by the tip of the spicule. The converse of this is seen later on when the arms diminish in length as the calcium carbonate of the pluteus skeleton is made use of by the developing urchin.

A third case is the lens of the vertebrate eye, which it is said depends for its formation upon a stimulus given by the optic vesicle or optic cup to the overlying ectoderm. This statement rests upon evidence brought forward by Spemann and more particularly by Lewis and Le Cron, that when (in the tadpole) the optic vesicle is experimentally injured or

destroyed, the lens is not developed, and that the lens may be developed from ectoderm taken from another part of the body or even from another tadpole, and grafted over the optic vesicle in place of the normal lens-forming cells, or from the ectoderm lying over an optic vesicle transplanted to some other region of the body.

Again, in tadpoles grown in certain solutions (sodium chloride, bromide, and nitrate) the optic vesicle may be some distance from the ectoderm, and the lens is then absent, and Stockard has found that in solutions of magnesium chloride a single median eye may be developed in embryos of the fish *Fundulus* instead of the two normal lateral eyes. Such median eyes have a lens which is obviously not developed from the ordinary lens-forming ectoderm.

It seems therefore quite clear that under certain conditions a lens may be formed over the optic vesicle but from other cells than those usually devoted to its formation. On the other hand it is urged that in certain cases lenses are undoubtedly developed from the usual cells and in the usual positions, in spite of the absence of the optic vesicle (King, Mencl, Stockard).

Spemann has found that the lens, if formed under these conditions, is small, but that by inserting an optic vesicle to replace that destroyed, a lens of full size is produced. An optic vesicle of *Bombinator* may even be used to replace one of *Rana*.

A fourth case is the formation of the cornea in Amphibia. Spemann and Lewis have both found that in the absence of the optic cup the corneal ectoderm does not 'clear' and that Descemet's membrane is not formed.

The development of the ear provides another instance. The auditory vesicle or membranous labyrinth is formed first and subsequently surrounded by the cartilage of the periotic or auditory capsule. The latter, of course, conforms to the shape of the former. The vesicle may be removed in an early stage and transplanted to another individual. Here it continues its development, and is surrounded by a capsule developed from connective tissue which would of course

otherwise have had a very different fate. The normal vesicle and capsule are developed alongside the transplant. It is even possible to graft a vesicle of *Rana* on to a larva of *Amblystoma*. The foreign vesicle then becomes enveloped in a periotic derived from *Amblystoma* cells. The normal *Amblystoma* labyrinth is present as well (Spemann, Lewis, Streeter).

Our last example is furnished by the experimental situs inversus viscerum et cordis produced by Spemann and Pressler in Amphibian larvae (*Rana* and *Bombinator*). A square piece is cut out of the back of the embryo in the medullary fold stage, turned right round so that its anterior end faces posteriorly and its right side to the left, and grafted in again. The part removed involves nervous system, notochord, some mesoderm, and the roof of the gut, but not the floor of the latter nor of course the heart or any subjacent structures. The embryo continues to develop, and shows inverted viscera and inverted heart. The part of the gut removed and inverted is in the region of the duodenum, but before the dorsal pancreas was developed; nevertheless, not only is the dorsal part of the intestine inverted, but the whole of the alimentary canal, that is, parts posterior and ventral to that which was turned round. In other words, the liver is pushed over to the left instead of to the right, the dorsal and ventral pancreas unite on the left instead of on the right, the stomach is on the right and the duodenum on the left, while the intestine passes into the rectum on the right. Moreover, the heart is inverted. The right is larger than the left vitelline vein, and the ventricle is bent to the left, the left auricle is larger than the right. The spiral valve starts at the right instead of at the left side of the truncus, so that the cavum aorticum is on the left, the cavum pulmonale on the right. The heart and viscera of the tadpoles operated on are the mirror images of the normal organs, the situs inversus viscerum et cordis is complete, and it is clear that an experimental alteration of the normal position of some parts has incited a corresponding change in other parts which were not immediately touched in the operation.

These examples are all taken from ontogeny. The study of Regeneration will give a few more. Thus in earthworms, new structures—heads and tails—are developed over the cut ends of the nerve-cord, and by exposing more than one cut surface of this part, the number of regenerated heads or tails may be correspondingly increased. So in the regenerating tail of vertebrata (Urodela). Injury to the nerve-cord starts a new formation, the cut end of this structure exerting apparently some stimulus upon surrounding tissues.

When aneurogenic limb buds of Amphibian embryos—i.e. limb buds removed before the ventral root (motor) nerves have grown into them—are transplanted on to strange positions in normal individuals, the posterior limb bud for instance on to the back of the head of another tadpole, they continue their development and acquire a normal nerve-supply to their normally differentiated muscles; the nerve-supply is derived from the nervous system of the host, the fibres growing into the graft and being guided in their course by the developing muscles of the latter (Harrison). The directive stimulus so exerted by one part upon another is seen again in the regenerating appendages of Insect larvae (Agrionidae), where the joints of the exoskeleton only appear after the insertion of the tendons of the newly differentiated muscle fibres.

Though the instances which at present can be cited of this interaction of parts to develop new structures in ontogeny and regeneration are lamentably few, they at least indicate the existence of a factor of the utmost importance; for it is clear that with this factor the whole process of development must be immensely simplified, though of course as truly determinate as if there were in the germ a unit representative of each separately inheritable character.

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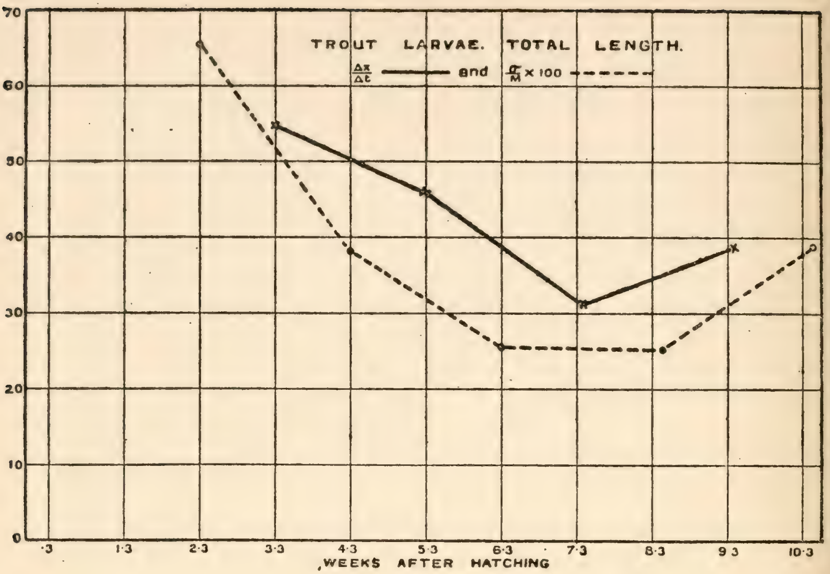
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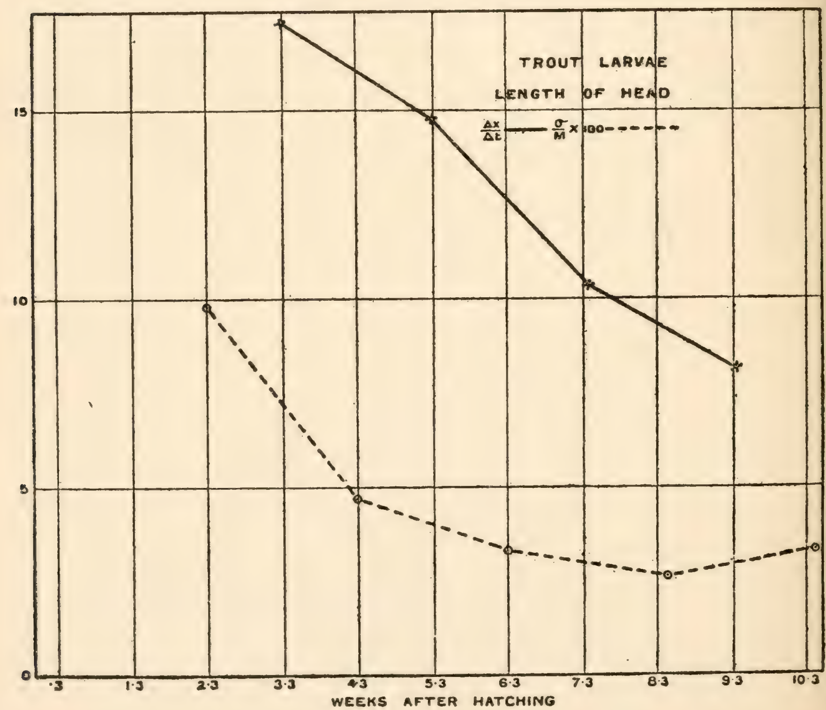
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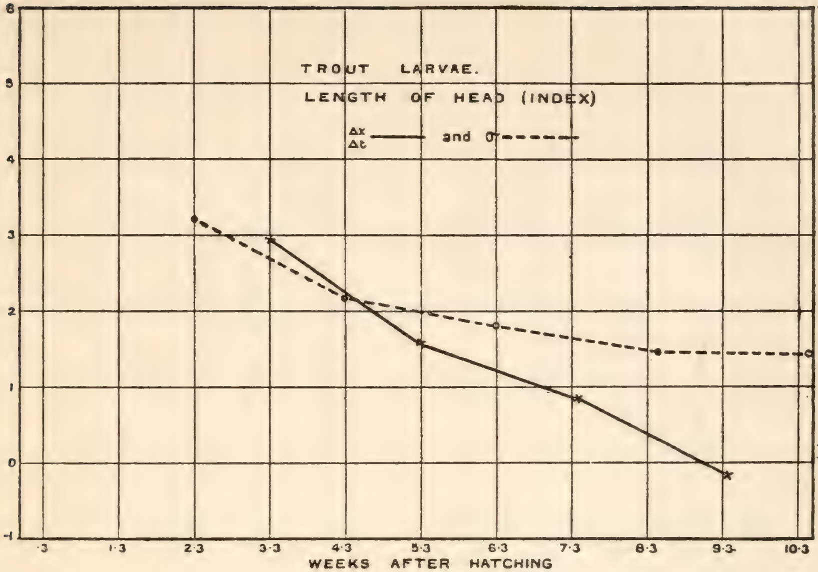
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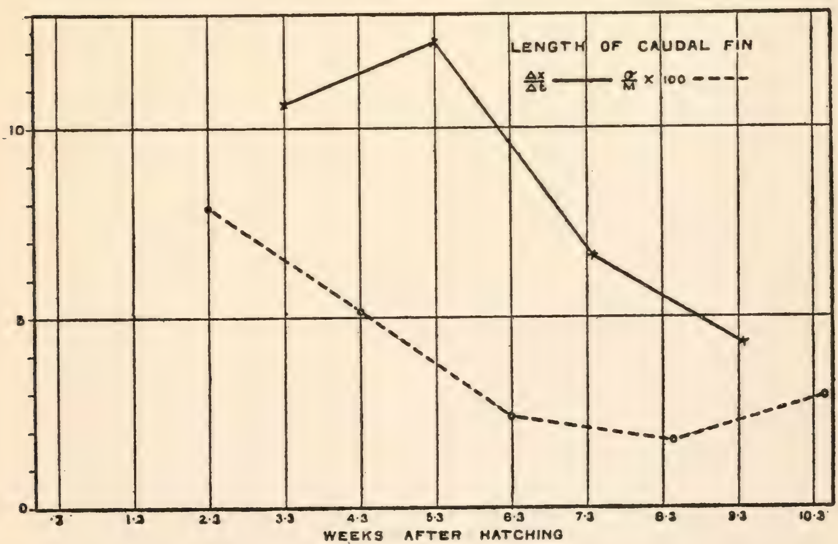
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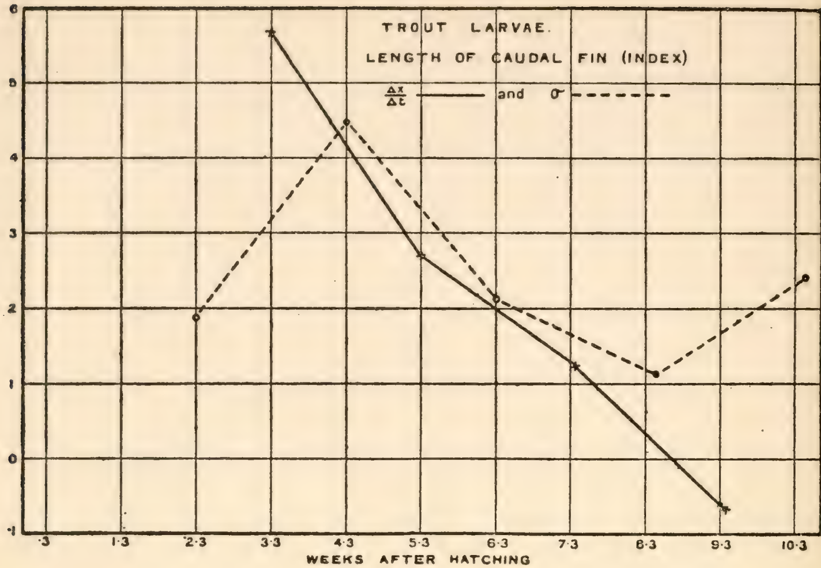
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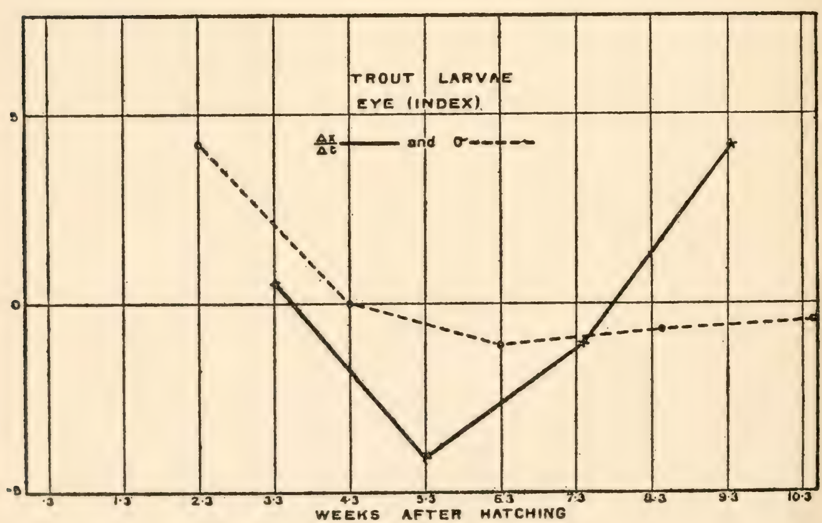
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